THE ROLE OF PLANT FUNCTIONAL TRAITS IN CENOZOIC ANGIOSPERM RADIATIONS

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Renske Onstein

ZUSAMMENFASSUNG

Es ist ein zentrales Ziel der Evolutionsbiologie, zu verstehen warum sich gewisse Organismengruppen in viele Arten diversifizieren, während die Diversifikation in anderen Gruppen, Zeiten und Orten viel langsamer vonstatten geht. Angiospermen, mit einer Diversität von ca. 250'000 Arten, sind ein exzellentes Taxon um dieses Phänomen zu untersuchen, da ihre Diversität sowohl räumlich als auch phylogenetisch ungleich verteilt ist. Diese Diversitätsunterschiede könnten von evolutionären Radiationen – der Vervielfältigung von Arten – herrühren, doch die Ursachen oder 'Auslöser' dieser Radiationen in Angiospermen sind noch unzureichend verstanden. Angiospermen verfügen über eine spektakuläre Vielfalt an morphologischen Formen und 'functional traits' (funktionelle Merkmale, z.B. Wuchsform, Blattgrösse, Blattform) und evolvierten infolgedessen eine breite Spanne von ökologischen Strategien, assoziiert mit verschiedenen Habitaten, Biomen und Vegetationstypen. Das wiederholte 'erfinden' von 'intrinsic traits' (innerer Merkmale) wurde daher als möglicher Antrieb der Angiospermen-Diversifikation vorgeschlagen.

In dieser Dissertation stelle ich die Hypothese auf, dass das Zusammenspiel von vegetativen 'functional traits' und Umwelt (z.B. Klima und Habitat) einige der spektakulären evolutionären Radiationen in Angiospermen während des Känozoikums (von vor ca. 66 Mio. Jahren bis jetzt) erklären könnte. Radiationen sind oft markant in den mediterranen Ökosystemen (MTEs, Mediterranean-type ecosystems) der Welt: der südafrikanischen Kap Region, Westaustralien, Kalifornien, Chile und dem Mittelmeerraum. Diese 'hotspots' enthalten etwa 20% der bekannten Gefässpflanzen, fast 50'000 Arten, in einem Gebiet das weniger als 5% der Erdoberfläche bedeckt. Obwohl sie sich geografisch getrennt auf verschiedenen Kontinenten befinden, teilen die fünf MTEs ein ähnliches Klima mit trockenen, heissen Sommern und kühlen, nassen Wintern. Diese Bedingungen könnten die Selektion für Arten mit kleinen, immergrünen Hartlaub-Blättern (sklerophyll), mit tiefem spezifischem Blattgewicht (SLA, specific leaf area) und einer Strauch-Wuchsform gefördert haben, was in analogen Vegetationstypen in den MTEs resultierte. Diese Merkmale können 'adaptiv' oder 'exaptiv' sein. Merkmale sind 'Adaptationen' wenn sie von der Umwelt natürlich selektioniert wurden und 'Exaptationen' wenn sie zuvor für eine bestimmte Funktion evolvierten (eine Adaptation), aber dann für einen neuen Zweck verwendet werden.

Hier nutze ich Methoden aus den Bereichen der 'functional trait' Ökologie und molekularen Phylogenetik und wende vergleichende phylogenetische Methoden an, um zu einem besseren Verständnis der 'functional traits'-Evolution zu gelangen. Ich benutze hierfür mediterrane Ökosysteme und mehrere Angiospermen-Clades als Studiensystem. Diese umfassen Penaceae, Phyliceae und Diosmeae (Kapitel II), Rhamnaceae (Kapitel III und IV), Proteaceae (Kapitel V) und Ericaceae, Fagales und Poales (Kapitel VI). Die räumliche Verbreitung der Arten in diesen Clades in verschiedenen Vegetationstypen und Biomen und deren intrinsische Variation in vegetativen 'functional traits' und ökologischen Strategien, macht sie ideal um diese Hypothese zu testen.

In Kapitel II untersuchte ich ob Veränderungen in Diversifikationsraten, 'functional traits' und Habitaten gemeinsam in den Phylogenien auftreten. Ich erstellte datierte Phylogenien für drei Kap-Clades (Penaceae, Phyliceae und Diosmeae) und zeigte in allen drei Clades parallel, dass Habitatswechsel von afromontanen Wäldern zu Fynbos mit Verringerung in Blattfläche und SLA assoziiert waren und entweder mit Erhöhungen der Diversifikationsrate zusammenfielen oder diesen vorausgingen. Des Weiteren sind Penaceae, Phyliceae und Diosmeae Arten typische Vertreter ihrer Vegetationstypen in Bezug auf ihre Merkmale. Ich argumentierte, dass die Expansion des Fynbos auf Kosten der Wälder im Miozän, angetrieben von Veränderungen im Feuerregime und der Aridifikation

des Kaps, eine ökologische Gelegenheit zur Diversifikation der Fynbos-Stammeslinien dargestellt haben könnte.

In Kapitel III testete ich die Hypothese, dass Kontinent-abhängige Artbildungs- und Aussterberaten zu Ungleichheiten in der Diversität der fünf MTEs dieser Welt geführt haben. Zu diesem Zweck erstellte und datierte ich Phylogenien für 280 Rhamnaceae Arten (27% der Gesamtartenzahl der Familie) und zeigte, dass Rhamnaceae-Stammeslinien in MTEs generell höhere Diversifikationsraten hatten als andernorts, dass aber Artbildungs- und Aussterbedynamiken ein Kontinent-abhängiges Muster aufwiesen. Hohe Artbildungs- und Aussterberaten wurden in kalifornischen Rhamnaceae Linien gefunden, sowie signifikant tiefe Aussterberaten in Rhamnaceae in MTEs des Kaps und Australiens. Diese Resultate deuteten auf unabhängige evolutionäre Geschichten von Rhamnaceae in MTEs hin, möglicherweise verbunden mit der Intensität von Klima-Oszillationen und der geologischen Geschichten der Regionen.

In Kapitel IV vertiefte ich die Resultate von Kapitel III und testete, ob die Kontinent-abhängigen Muster von Artbildung und Aussterben in MTEs mit der Evolution von sklerophyllen (hartlaubigen) und nicht-sklerophyllen Merkmalssyndromen in diesen Regionen verbunden sein könnten. Diese Merkmalssyndrome beinhalteten artspezifische Daten über SLA, Blattgrösse, Spineszenz, Blattphänologie, Wuchsform und Blattrand-Typ. Resultate legen nahe, dass die Evolution von Sklerophyllie (Hartlaubigkeit) zu erhöhten Diversifikationsraten in Rhamnaceae-Linien in den MTEs des Kaps und Australiens beigetragen hat, indem die Reduktion der Aussterberaten evolutionäre Beständigkeit vereinfachte. Die historisch relativ stabilen Bedingungen am Kap und in Australien sind konsistent mit dieser Beständigkeits-Hypothese. Des weiteren wurde die morphologische Konvergenz in MTEs lange als Adaptation an die klimatischen Ähnlichkeiten dieser Regionen interpretiert; allerdings zeigte ich, dass diese Merkmalssyndrome wahrscheinlich vor dem sommerdürrem Klima in den MTEs evolvierten, womit sie nicht für dieses Selektionsregime adaptiv sein können. Nichtsdestotrotz, Hartlaubigkeit evolvierte zeitgleich mit dem Habitatswechsel ans Kap und in die australischen MTEs und könnte somit potentiell eine Adaptation an die für diese Region typischen Bodenbedingungen sein.

In Kapitel V testete ich die Voraussage, dass die Evolutionsraten von 'functional traits' und klimatischen Nischen während einer Radiation gekoppelt sind. Hierfür erstellte ich eine datierte Phylogenie für 337 Proteaceae-Arten (21% der Gesamtartenzahl der Familie) und sammelte Daten für 'functional traits' von Blättern (Blattfläche, Hartlaubigkeit, Blattform) und die klimatischen Nischen von 261 bzw. 1645 Arten. Die Resultate wiesen darauf hin, dass stabilisierende Selektion der Auslöser gewesen sein könnte, dass Stammeslinien die in offenen Vegetationstypen evolvierten sich auf Merkmals- und klimatische Nischen-Optima diversifiziert haben, welche sich von denen in geschlossenen Vegetationstypen unterscheiden. Ausserdem waren die Evolutionsraten von Merkmalen und klimatischen Nischen stark korreliert, und diese Raten waren besonders hoch in Clades die in offener Vegetation vorkommen. Ich argumentierte, dass die Einwirkung variabler Mikroklimate in offenen Landschaften wie denen in MTEs, die ansonsten durch geschlossene Bewaldung gedämpft werden, höhere innerartliche Variabilität der Merkmale begünstigen könnte, was Radiationen in diesen Systemen vereinfacht hätte.

In Kapitel VI entwickelte ich ein konzeptuelles Gerüst um intrinsische und extrinsische Variablen welche in einer Radiation involviert sind zu klassifizieren. In den Testgruppen Ericaceae, Fagales und Poales fand ich dreizehn Veränderungen in Diversifikationsregimes (d.h. Radiationen) und stellte fest, ob die assoziierten Variablen vor der relevanten Radiation (Hintergrundvariablen), gleichzeitig mit der Radiation (Auslöser) oder später (Modulator) evolvierten. Die Resultate legten nahe, dass Radiationen sowohl extrinsische Voraussetzungen als auch intrinsische Merkmale benötigen, aber dass die Abfolge derselben nicht wichtig ist. Diversifikations-Treiber zeichnen sich dadurch aus, dass sie innerhalb der Radiation variabler sind als konservierte

Merkmale die lediglich die Belegung eines neuen Habitats ermöglichen. Dieses konzeptuelle Gerüst vereinfacht die Untersuchung von kausativen Faktoren von evolutionären Radiationen.

Die Forschungsarbeit in dieser Dissertation betont das komplexe Zusammenspiel zwischen biologischer Diversität, morphologischer Form und der globalen Umwelt. Das Aufdecken der Innovation und Evolution von 'functional traits' und deren Rolle in Angiospermen-Diversifikation kann viel zu einem erhöhten Verständnis der gegenwärtigen Vielfalt und Verbreitung von Arten beitragen, genauso auch zum Aufzeigen der Konsequenzen der ökologischen Dominanz gewisser funktioneller Typen, und damit funktioneller Diversität in bestehenden Ökosystemen.

SYNOPSIS

It is a central goal in evolutionary biology to understand why some groups of organisms diversify into many species, while diversification is much slower in other groups, times and places. Flowering plants (angiosperms), with a standing diversity of ca. 250.000 species, is an excellent taxon to investigate this phenomenon, as their diversity is unevenly distributed, both spatially and phylogenetically. These diversity discrepancies may have resulted from evolutionary 'radiation' – the multiplication of species – but the causes or 'triggers' of radiations in angiosperms remain poorly understood. Angiosperms display a spectacular variety of morphological forms and 'functional traits' (e.g. growth forms, leaf sizes, leaf shapes) and have consequently evolved a wide range of ecological strategies associated with different environments, biomes and vegetation types. The repeated 'innovation' of intrinsic traits has therefore been proposed as a possible driver of diversification in angiosperms.

In this thesis, I hypothesize that the interaction between vegetative functional traits and environments (e.g. climate and habitat) may explain some of the spectacular evolutionary radiations in angiosperms during the Cenozoic (ca. 66 Ma till present). Radiations are often prominent in the Mediterranean-type ecosystems (MTEs) of the world: the Cape, Western Australia, California, Chile and the Mediterranean Basin. These 'hotspots' contain about 20% of the known vascular plant species, almost 50'000, in an area which covers less than 5% of the Earth's surface. Although they are geographically separated on different continents, the five MTEs share a similar climate of dry, hot summers and cool, wet winters. These conditions may have selected for species with small, evergreen, sclerophyllous leaves, with a low specific leaf area (SLA) and a shrubby growth form, resulting in analogous vegetation types among MTEs. These traits may be 'adaptive' or 'exaptive'. Traits are 'adaptations' if they are naturally selected for by the environment and 'exaptation' if they have previously evolved for a particular function (an adaptation), but are coopted for a new use.

Here, I used methodologies from the fields of functional trait ecology and molecular phylogenetics and I applied phylogenetic comparative methods to contribute to a better understanding of functional trait evolution and the role of traits in evolutionary radiation, by using Mediterranean-type ecosystems and several angiosperm clades as study systems. These include Penaeaceae, Phyliceae and Diosmeae (chapter II), Rhamnaceae (chapters III and IV), Proteaceae (chapter V) and Ericaceae, Fagales and Poales (chapter VI). The spatial distribution of the species in these clades in different vegetation types and biomes, and their intrinsic variation in vegetative functional traits and ecological strategies, makes them ideal to test the hypotheses.

In chapter II I investigated the co-occurrence of shifts in diversification rates, functional traits and habitats on phylogenetic trees. I built dated phylogenetic trees for three Cape clades (Penaeaceae, Phyliceae and Diosmeae) and showed that afromontane forest to fynbos shifts were associated with decreases in leaf area and SLA and preceded or coincided with increases in diversification rates in a parallel fashion. Furthermore, Penaeaceae, Phyliceae and Diosmeae species are typical members of their vegetation types in terms of their traits. I argued that expansion of the fynbos at the cost of forest in the Miocene, driven by changes in fire regime and aridification in the Cape, may have provided an ecological opportunity for the diversification of fynbos lineages.

In chapter III I tested the hypothesis that continent-dependent speciation and extinction rates have led to disparity in diversity between the five MTEs of the world. To this end I built and dated phylogenetic trees for 280 Rhamnaceae species (27% of total number of species in the family) and demonstrated that Rhamnaceae lineages in MTEs generally showed higher diversification rates than elsewhere, but speciation and extinction dynamics showed a pattern of continent-dependence. High speciation and extinction rates were detected in Californian Rhamnaceae lineages and significantly

low extinction rates in Rhamnaceae occurring in Cape and Australian MTEs. These results indicated independent evolutionary histories of Rhamnaceae in MTEs, possibly related to the intensity of climate oscillations and the geological history of the regions.

In chapter IV I elaborated the results from chapter III, and tested if the continent-dependent pattern of speciation and extinction in MTEs may be associated with the evolution of sclerophyllous and non-sclerophyllous trait syndromes in these regions. These trait syndromes included species-specific data on specific leaf area, leaf size, spinescence, leaf phenology, growth form and leaf margin type. Results suggested that the evolution of sclerophylly has contributed to increased diversification rates of Cape and Australian Rhamnaceae lineages, by reducing extinction rates, and thereby facilitating evolutionary persistence. The historical relatively stable conditions in the Cape and Australia are consistent with this persistence hypothesis. Furthermore, the morphological convergence in MTEs has long been interpreted as adaptation to climatic similarities among these regions; however, I demonstrated that these trait syndromes have likely evolved prior to summer-drought climates in MTEs, thereby failing to be adaptive to this selective regime. Nevertheless, sclerophylly evolved contemporaneously with the transitions to Cape and Australian MTEs, and may therefore potentially be an adaptation to edaphic conditions typical of these regions.

In chapter V I tested the prediction that rates of functional trait evolution and climatic niche evolution are coupled during radiation. To this end, I built a dated phylogenetic tree for 337 Proteaceae species (21% of total number of species in the family) and collected leaf functional trait data (blade area, sclerophylly, leaf shape) and climatic niche data for 261 and 1645 species respectively. Results indicated that stabilizing selection may have triggered lineages which evolved in open vegetation types to diversify towards trait and climatic niche optima distinct from those which evolved in closed vegetation types. Furthermore, the rates of trait and climatic niche evolution were strongly correlated, and these rates were particularly high in clades occurring in open vegetation. I argued that the exposure to variable micro-climates in open landscapes such as those in MTEs, which are otherwise buffered by closed forest covers, may favour higher interspecific trait variability, which may have facilitated the radiations in these systems.

In chapter VI I developed a conceptual framework to classify intrinsic and extrinsic variables involved in radiation. Using Ericaceae, Fagales and Poales as test cases, thirteen shifts in diversification regimes (i.e. radiations) were detected and I determined whether the associated variables originated before the relevant radiations (backgrounds), simultaneously with the radiations (triggers), or evolved later (modulators). These results suggested that radiations require both extrinsic conditions and intrinsic traits, but the sequence of these is not important. Diversification drivers can be identified as being more variable within a radiation than conserved traits that only allow occupation of a new habitat. This framework may facilitate exploration of the causative factors of evolutionary radiations.

The research in this thesis emphasizes the intricate interplay between biological diversity, morphological form, and the global environment. Revealing the roles of innovation and evolution of functional traits in angiosperm diversification can contribute much to an increased understanding of current species diversity and distribution, as well as reveal the consequences for ecological dominance of certain functional types, and thus functional diversity, in extant ecosystems.

CHAPTER I: GENERAL INTRODUCTION

Diversification and innovation

A long standing question in evolutionary biology is why some groups of organisms diversify into many species, while diversification is much slower in other groups, times and places (e.g. Dobzhansky 1950, Simpson 1953, Gould and Eldredge 1977, Sepkoski 1979, Cardillo 1999, Wiens and Donoghue 2004, Mittelbach et al. 2007, Ezard et al. 2011). Flowering plants (angiosperms), with a standing diversity of ca. 250.000 species, are excellent to investigate this question in, as their diversity is unevenly distributed, both spatially and phylogenetically. Furthermore, angiosperms are primary producers and dominate the vegetation of most modern terrestrial ecosystems and biomes, making them one of the main drivers of ecosystem functioning worldwide (Midgley 2012). The extraordinary species richness of angiosperms is also matched by exceptional – vegetative and reproductive – structural diversity, with growth forms ranging from minute floating aquatics and tiny herbs to massive woody forest trees, and leaves from broad, highly dissected, lobed leaves to tiny needle-like leaves. This variation in functional types and traits, i.e. structures that affect plant fitness through their effects on growth, survival and reproduction (Violle et al. 2007), underpins their ecological success. In addition, angiosperm diversity is exceptional in comparison to their species-poor sister lineage, the gymnosperms with ca. 1'050 extant species, despite having had equal amounts of evolutionary time to diversify. Understanding their diversification – the balance between speciation and extinction – is therefore essential to understanding how diversity is distributed across the Earth's surface (Mittelbach et al. 2007), the Tree of Life (Alfaro et al. 2009, Rabosky 2014), and over geological time (Ezard et al. 2011). Nevertheless, the drivers of diversification or 'shifts' in the diversification rate (e.g. Alfaro et al. 2009) remain enigmatic. Crepet and Niklas (2009) hypothesize that the most plausible interpretation for the success of angiosperms is that they continued to evolve morphological and reproductive innovations and 'reinvented' themselves whereas this reinvention did not happen in other groups of plants (e.g. pteridophytes and gymnosperms). This may have led to 'episodes' of angiosperm diversification. In this thesis, I will refer to these episodes of high diversification as 'radiations' – i.e. the multiplication of species ('explosive speciation' sensu Givnish 2010). Revealing the innovation and evolution of functional traits and their role in angiosperm radiations can contribute much to an increased understanding and maintaining of current species diversity and distribution as well as reveal adaptations to climatic change. In this thesis I aim to reveal the intimate dependence of diversity on climate and functional trait innovation. This emphasizes the intricate interplay between biological diversity, morphological form, and the global environment.

Angiosperm diversification through time

Early angiosperm diversification

Based on fossil evidence (Friis et al. 2006) and molecular dating (Magallón et al. 2015), angiosperms started diversifying in the Early Cretaceous, 135-130 million years ago (Ma), and half of the extant families originated in the Cretaceous. Early flowering plants may have been represented by a highly speciose clade of weeds (Wing and Boucher 1998), but may as well have been woody forest understory species (Feild et al. 2004).

The enigmatic success of angiosperms has been linked to one or more of the unique innovations shared by all angiosperms, such as closed carpels, vessels, and an increased growth rate (compared to gymnosperms), but the rise and dominance of angiosperms is unlikely to be linked to a

single innovation at the time of origin. Because, first, there was no shift in diversification rate detected at the base of the angiosperms (Sanderson and Donoghue 1994), second, angiosperm diversity is unevenly distributed among clades, suggesting diversification rate shifts among subclades and third, early in their respective evolutionary histories, angiosperms, gymnosperms and pteridophytes all experienced comparable high diversification rates (Crepet and Niklas 2009). The difference between these groups is that in gymnosperms and pteridophytes diversification rates slowed down over time, whereas angiosperms show episodes or 'bursts' of high diversification.

Cenozoic angiosperm diversification

Most extant angiosperm diversity evolved during the Cenozoic (66 Ma till present), a period characterized by dramatic global climate change (Zachos et al. 2001, Zachos et al. 2008). From the Cretaceous, global temperatures gradually increased, reaching a peak during the Early Eocene Climatic Optimum, after which the climate became cooler until the dramatic cooling near the Eocene-Oligocene boundary at 33 Ma. The Early Miocene was characterized by a temperature increase, and the mid-Miocene (14 Ma) initiated the 'modern world' in which climate became gradually cooler and more seasonal (Potter and Szatmari 2009). These fluctuations were identified by global deep-sea oxygen and carbon isotope records (Zachos et al. 2001). These climate fluctuations evidently correlate with changes in the global distribution of biomes and vegetation types. These changes are documented from a large number of fossil sites, using techniques such as the nearest-living-relative (NLR) method to link fossil taxa to extant taxa and physiognomical methodologies by using extant relationships between traits and climate. These have led to improved palaeoenvironmentral reconstructions (Mosbrugger et al. 2005, Kvaček 2007, Eldrett et al. 2009).

These climate and vegetation changes may have had a major impact on angiosperm diversification, and may be responsible for the episodic 'discrete' character of Cenozoic angiosperm diversification. These episodes, or radiations, are consistent with the punctuated equilibria model (Gould and Eldredge 1977), and suggest that diversity is discrete rather than gradually accumulated through time. However, Cenozoic neotropical fossil floras do not give an indication of pulsed changes in composition, but rather gradual changes correlated to global temperature fluctuations (Jaramillo et al. 2006).

Red Queen and Court Jester in angiosperm diversification

Several models have been developed to explain the process of diversification and species richness through time, such as the Red Queen and Court Jester models of evolution. The Red Queen model (Van Valen 1973) follows Darwin's initial ideas that evolution results from the balance of biotic pressures. *Intrinsic traits*, such as physiological tolerance or 'adaptability' to hard times, are the main dimensions in this model (Benton 2009). This model is characterized by continuous evolution, speciation and extinction rates are constant, and species do not evolve towards better adapted forms. Instead, "it takes all the running you can do, to keep in the same place" (Red Queen to Alice in Lewis Carroll's *Through the Looking-Glass*), in which species need to compete with a constantly changing biotic environment. The Court Jester model (Barnosky 2001), however, regards *extrinsic processes* related to physical-environmental perturbations, such as climate change, as most important for driving major changes in organisms and ecosystems. The model refers to the 'waxing and waning' of clades in response to mass extinction events, and turnover of species due to fluctuating origination and extinction rates. However, the Red Queen and Court Jester models may not be mutually exclusive, and they may operate predominantly over different geographic and temporal scales (Barnosky 2001, Benton 2009), as competition, predation and other biotic factors may shape ecosystems more locally and over short time spans, whereas regional and global patterns may be shaped by extrinsic climate, oceanographic and tectonic events in deep time.

Traits in the Red Queen model seem to function as a 'tool' for organisms to persist (and compete), rather than functioning as 'innovations' that could lead to radiations as in Crepet and Niklas' (2009) model. Although the Court Jester model does not explicitly state the role of intrinsic traits during evolutionary change, the idea of discontinuous speciation and extinction rates does match the character of radiations and 'depauperons' (species-poor lineages) (Donoghue and Sanderson 2015) for angiosperms (Magallon and Sanderson 2001, Magallon and Castillo 2009, Xing et al. 2014). This indicates that angiosperm diversification, at least at the macro-evolutionary scale, may primarily follow a Court Jester model to evolution, in which the global abiotic environment stimulates diversification rate shifts. Nevertheless, the response of lineages to climate change, in terms of adaptation, speciation and extinction, may very likely be dependent on their intrinsic traits and innovations.

Cradles and museums in angiosperm diversification

We can classify angiosperm radiations during the Cenozoic into two groups: recent and rapid radiations *versus* mature radiations (modified from Linder 2008). Recent and rapid radiations typically have short branches as detected on phylogenetic trees, high speciation rates and happened during the Plio-Pleistocene, such as radiation of the South-American legume genus *Inga* (Richardson et al. 2001a), the South African Aizoaceae (Klak et al. 2004) and the Andean *Lupinus* (Hughes and Eastwood 2006). They can drive 'cradles' to diversity if restricted to a certain area, such as a tropical rainforest (Stenseth 1984). Mature radiations may span a longer time scale, can be initiated in the Eocene, Oligocene or Miocene, can have a combination of short and long branches, and may be typically driven by low extinction rates. An example is the African Restionaceae (Linder 2008). These radiations are typically following a 'museum' model to diversity, in which time to accumulate lineages and low extinction rates are driving diversity patterns (Stenseth 1984). Extant diversity is most likely the result of a mix of recent, rapid and mature radiations. The explosion of modelling tools to understand diversification based on molecular phylogenies and the distribution of branch-lengths (Morlon 2014) has made it possible to detect changes in diversification rates (Alfaro et al. 2009, Rabosky 2014) and signatures of speciation and extinction (Nee et al. 1994, Crisp and Cook 2009, FitzJohn et al. 2009, FitzJohn 2012) even with a limited fossil record.

Correlates of angiosperm diversification

The Simpsonian model to angiosperm diversification

The idea of repeated innovation (Crepet and Niklas 2009) leading to radiations may be associated with entering a new 'adaptive zone' (Simpson 1953, p: 201-202): "[…] representing a characteristic reaction and mutual relationship between environment and organism, a way of life and not a place where life is led". Simpson refers here to the close interaction between organism and environment, in which one (the organism) cannot be seen independently from the other (the environment). For entering a new adaptive zone in which a lineage can expand taxonomically, structurally and/or ecologically, three conditions need to be satisfied: physical access to this zone (e.g. through dispersal in case of novel environments), the evolution of appropriate traits that allow the organism to occupy novel environments and to interact with existing environments in a novel way (e.g. innovation following Crepet and Niklas [2009]), and the zone should either be empty, or the occupants are competitively inferior. The Simpsonian model emphasizes the roles of *both* intrinsic and extrinsic variables during radiation leading to taxonomic and phylogenetic (and possibly structural and ecological) diversity discrepancies (Guyer and Slowinski 1993).

Trait-dependent diversification

The occurrence of discrete radiation in angiosperms thus suggests that this success may be linked to episodes of functional trait innovation. In the past, much emphasis was laid on intrinsic variables related to the reproductive attributes of the plants, such as flower and fruit traits (Hodges and Arnold 1995b , Hodges 1997, Crepet and Niklas 2009 for many examples), and the mutually beneficial animal-plant relationships may have led to increased diversification. A classic example is the evolution of nectar spurs in columbine flowers in association with pollinator tongue lengths. These pollinators and their variation in tongue lengths were shown to provide the potential for columbine species to evolve towards adaptive peaks predefined by pollinator morphology (Whittall and Hodges 2007). Pollinator-specificity may enforce reproductive isolation and divergence between columbine populations, ultimately causing speciation. Evidently, the role of the biotic environment – i.e. the availability of and the interaction with pollinators and dispersers – has been of direct influence in the evolution of reproductive traits in angiosperms, and their effect on diversification rates.

Vegetative, functional traits may also play an important role in angiosperm diversification (e.g. Boucher et al. 2012, Drummond et al. 2012b, Verdú and Pausas 2013). Although these traits probably do not have a direct effect on the construction of reproductive isolation, they evidently affect the fitness of plants through their effects on growth and survival (Violle et al. 2007), and may consequently influence diversification. Lineages with the 'right' set of traits may for example persist in the environment over longer time and diversify by having relatively low extinction rates (Crepet and Niklas 2009, Ezard et al. 2011). Furthermore, the evolution of vegetative traits may allow lineages to occupy new environments. For example, the shift from annual to perennial may have been the innovation causing 'ecological release' in the Andean *Lupinus*, allowing them to consequently evolve a disparity of growth forms during their radiation (Drummond et al. 2012b, Hughes and Atchison 2015). Furthermore, traits related to the life-history of plants can influence diversification rates through effects on generation times (Smith and Donoghue 2008b, Bromham et al. 2015), in which populations with short, non-overlapping generations may have relatively fast diversification rates by increasing the probability of fixation of genetic novelties associated with each generation (Thomas et al. 2010). Consistent with this idea, relatively low rates of molecular evolution were detected in woody versus herbaceous angiosperm lineages (Smith and Donoghue 2008b), but diversification rates between lineages which differ in their strategies in response to fire, i.e. resprouters (overlapping generations) *versus* obligate seeders (non-overlapping generations), were not detected to be significantly different (Verdú et al. 2007).

Importantly, and similarly to reproductive traits, the evolution of vegetative, functional traits and their effect on diversification rates seems to be dependent on the (abiotic) environment in which a lineage evolves. Traits are called 'adaptations' if they are naturally selected for by the environment and 'exaptation' (Gould and Vrba 1982) refers to when a trait "previously shaped by natural selection for a particular function (an adaptation), is coopted for a new use" (Gould and Vrba 1982). An example of an exaptation – although difficult to test – are feathers, which initially evolved for heat regulation, were coopted for display, and later coopted for use in bird flight (Gould and Vrba 1982).

Environment-dependent diversification

In addition to intrinsic innovation, the Simpsonian model suggests that diversification rates may be affected by the extrinsic 'opportunities' of new geographical areas, environments, or biomes encountered by lineages (e.g. Baldwin and Sanderson 1998, Moore and Donoghue 2007, Drummond et al. 2012b). Previous studies corroborated this idea. For example, increased rates of diversification in the Dipsacales were correlated with movement into new geographic areas (Moore and Donoghue 2007), the invasion of montane ecosystems in *Lupinus* (Drummond et al. 2012b), and the colonisation of Hawaii by the Hawaiian silverswords (Baldwin and Sanderson 1998). The ecological opportunities

associated with new environments may influence diversification rates by changing the selective regimes acting on natural populations, ultimately causing disruptive (diversifying) selection (Yoder et al. 2010). These selective regimes could be related to the variability (heterogeneity) of the environment, such as climatic niche heterogeneity. Indeed, rates of climatic niche evolution were shown to be correlated to rates of species diversification in amphibians (Kozak and Wiens 2010), primroses (Evans et al. 2009) and in Cape *Babiana* (Iridaceae) (Schnitzler et al. 2012). Other examples include pollinator niches, such as those important for the columbine radiation (Whittall and Hodges 2007), and soil type niches, which triggered diversification in several plant genera in the Cape Floristic Region (CFR) of South Africa (Schnitzler et al. 2011).

The interaction between traits and environments

Following Simpson's model, phenotypic modifications – i.e. morphological or physiological change, a set of traits, or a 'trait syndrome' (Stebbins 1974, Reich et al. 2003, Verdú and Pausas 2013) – can potentially enable organisms to survive in new environments, and are therefore essential for radiation. However, such change is thought to be rare with lineages usually being ecologically conserved (Prinzing 2001) and consequently biome shifts in plants are generally infrequent (Crisp et al. 2009). The evolution of the required ecophysiological and morphological attributes to survive in new environments should therefore either precede (e.g. Ackerly 2004a) or coincide (e.g. Schmerler et al. 2012) with the transition to the new environment. For instance, the innovation of the CAM-succulence syndrome and C4-photosynthesis were pre-requisites for colonization of arid habitats in C4-grasses and cacti, but the shift in diversification rate happened later, possibly in response to the expansion of these arid habitats in the Late Miocene-Pliocene (Sage 2004, Arakaki et al. 2011). Similarly, the innovation of proteins for freeze avoidance in Antarctic fishes allowed the exploitation of new environments created by increased glacial and ice sheet activity in the Late Miocene, resulting in an increase in the diversification rate (Near et al. 2012). Thus, species diversity patterns, caused by changes in diversification rates, may be the result of the evolution of a trait syndrome facilitating the occupation of new habitats (backgrounds, triggers), but these traits and habitats may not always be sufficient to explain the process of speciation within the radiation (modulators) (sensu Bouchenak-Khelladi et al. 2015). These results suggest a close relationship between plant functional traits, palaeo-environmental change, and diversification rates.

Mediterranean-type ecosystems as a study system

If radiations are bounded to certain geographical areas or environments, they can create species richness discrepancies, or 'diversity anomalies' (Jiménez and Ricklefs 2014), between environments. In addition, immigration and emigration rates into and out of environments, and the time available for an environment to accumulate diversity, will affect species richness in the environment. The latitudinal diversity gradient indicates that species richness is highest in the tropics, and decreases towards the poles (Mittelbach et al. 2007). Global spatial predictors of vascular plant species richness include potential evapotranspiration, the number of wet days per year, and measurements of topographical and habitat heterogeneity (Kreft and Jetz 2007). One of the exceptions to the diversity gradient is the Mediterranean biome, which shows exceptional species richness and endemism, particularly compared to other temperate regions and adjacent subtropical regions (Cowling et al. 1996, Kreft and Jetz 2007).

The five Mediterranean-type ecosystems of the world

"Sowohl bei mehreren endemischen Gattungen des Caplands, als solchen, welche sonst nur vereinzelt im tropischen Afrika oder im Mediterrangebiet auftreten, nehmen wir mehrfach die Eigenthümlichkeit wahr, dass sie im Capland eine ausserordentlich grosse Anzahl localisirter Arten entwickeln [...] Diese Vielgestaligkeit ist also nicht abhändig von dem Ursprung der gattungen, sondern der Beschaffenheit des Landes. Auch hier ist es, wie in Australien, ein im Sommer trocknes Gebiet, das den Polymophismus in so hohem Grade begünstigte. [...] Es haben also hier wesentlichen klimatische Aenderungen seit langer Zeit nicht stattfinden können."

Translation:

"In several endemic genera of the Cape, as well as those which only sporadically occur in tropical Africa and the Mediterranean region, we repeatedly observe the peculiarity that they evolve in the Cape in an extraordinarily large number of localized species [...]. This diversity is therefore not conditional on the origin of the genus, but the nature of the country. Again, it is, as in Australia, a summer-dry area that favours such a high degree of polymorphism. [...] Also here, significant climatic changes could not have taken place for a long time."

Adolf Engler (1882, p. 284-285) in "*Versuch einer Entwicklungsgeschichte der extratropischen florengebiete der Südlichen hemisphäre und der Tropischen gebiete*."

The five Mediterranean-type ecosystems (MTEs) of the world (the southern African Cape, California-Baja California, the Mediterranean Basin, central Chile and South and Southwest Australia) are classified as biodiversity conservation 'hotspots' (Mittermeier et al. 2004). Together they contain about 20% of the known vascular plant species, almost 50'000, in an area which covers less than 5% of the Earth's surface. Although the MTEs are geographically separated on different continents, they are characterized by similar climatic conditions of generally dry, hot summers and cool, wet winters (Aschmann 1973, Castri 1973, Kottek et al. 2006). Nevertheless, the processes which triggered the high *in-situ* diversity in MTEs remain enigmatic (e.g. Linder 2003, Lancaster and Kay 2013), but could be high immigration rates, long time to accumulate diversity in the systems, or evolutionary radiation (Sauquet et al. 2009, Valente et al. 2010a, Buerki et al. 2012, Buerki et al. 2013, Lancaster and Kay 2013). This system therefore provides the ideal situation to investigate evolutionary radiations, and the roles of traits, habitats and climate during radiation.

Traits in Mediterranean-type ecosystems

The comparable climatic conditions in MTEs may have selected for plants with similar functional traits, resulting in analogous vegetation types (Schimper 1903, Specht 1979, Cowling et al. 1996). Mediterranean vegetation includes predominantly dry shrub- or heathland, but sclerophyllous or drought-deciduous forests or woodlands, afromontane forest along rivers (in the CFR) and semi-succulent shrublands can be found as well (Fig. 1) (Cowling et al. 1996). The functional traits typical for the shrubland vegetation are a woody, shrubby growth form, with often small, evergreen, sclerophyllous leaves, and fire-adaptations (Schimper 1903, Specht and Moll 1983). Sclerophyllous leaves are associated with a relatively low photosynthetic capacity, a high proportion of leaf stored carbon, low leaf-nitrogen concentrations and a low ratio between leaf area and mass (low specific leaf area, SLA) (Wright et al. 2004). These traits can provide an advantage under water-stress (i.e. summer-drought) conditions, herbivory-stress and / or nutrient-poor conditions (Fonseca et al. 2000, Wright et al. 2004).

Interestingly, there is a strong indication that sclerophyllous traits evolved in pre-Mediterranean (sub)-tropical ancestors of Mediterranean lineages in California and the Mediterranean Basin, and was ecologically 'filtered' into these MTEs when these arose in the Plio-Pleistocene, suggesting that they are pre-adaptations or exaptations to summer-drought (Herrera 1992, Verdú et al. 2003, Ackerly 2004a, Sniderman et al. 2013). Furthermore, Verdu and Pausas (2013) showed that functional traits affected diversification rates of lineages in the Mediterranean Basin after the onset of the Mediterranean climate ca. 3.6 Ma. These results stress the importance of history and trait evolution in understanding spatial 'convergence' (Schimper 1903) and the dominance of certain ecological strategies in a system.

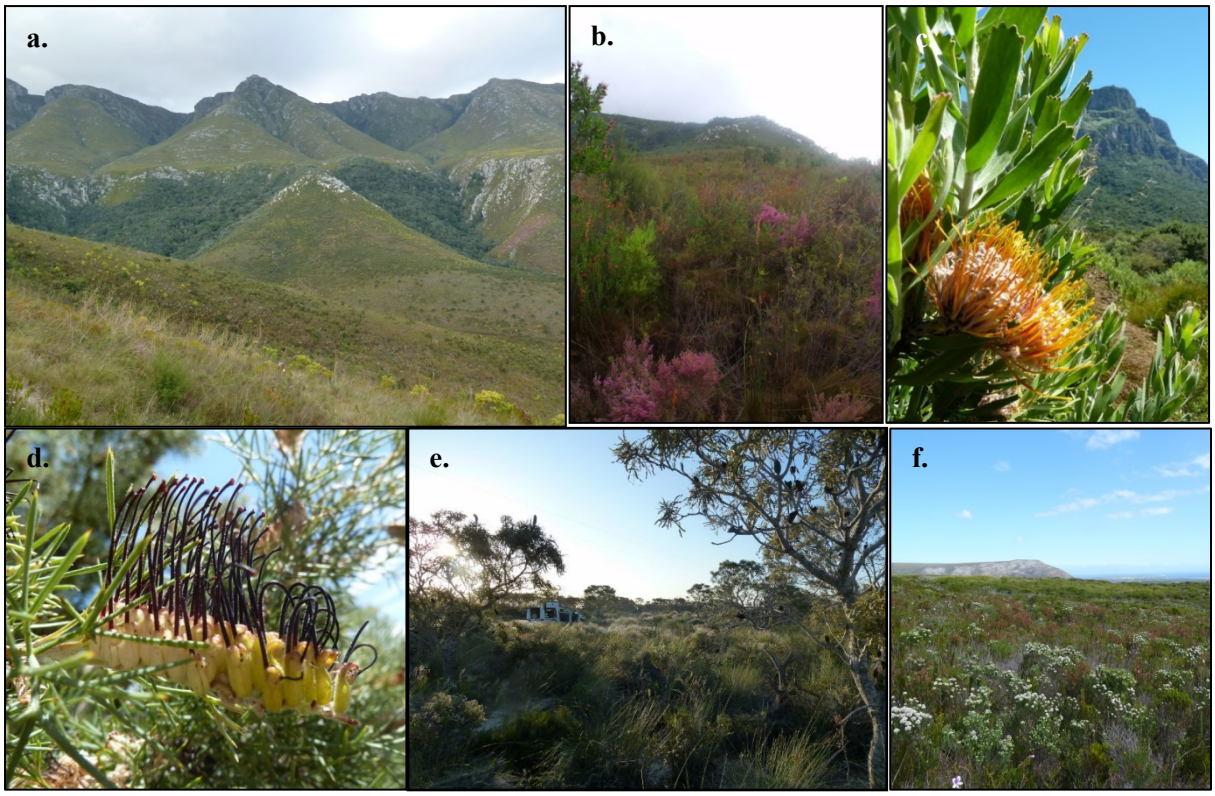

Figure 1
Vegetation types and species in Mediterranean-type ecosystems. a. Fynbos and afromontane forest in the Cape Floristic Region (CFR), b. fynbos in the CFR, c. *Leucospermum erubescens* (CFR), *d. Grevillea armigera* (Southwest Australian Floristic Region, SWAFR), e. Kwongan in SWAFR, f. coastal fynbos in CFR.

Climatic niches in Mediterranean-type ecosystems
There is no consensus concerning the processes which could have 'triggered' and 'modulated' (Bouchenak-Khelladi et al. 2015) radiations in Mediterranean-type ecosystems (e.g. the Proteaceae radiation, Sauquet et al. 2009). Several hypotheses stress the importance of environmental heterogeneity in the system (Linder 2003, Hopper and Gioia 2004), which could have partitioned niches and influenced diversification rates through the process of disruptive selection (Linder 2005). The niche of a species can be defined as the set of biotic and abiotic conditions that allow a species to maintain a viable population (Hutchinson 1957). The niche therefore consists of several 'dimensions' and diversification in Mediterranean-ecosystems (e.g. the Cape) has been linked to some of these dimensions, such as climatic niches (Carlson et al. 2011, Schnitzler et al. 2012), pollinator niches (Johnson 1996) and soil type niches (Schnitzler et al. 2011). Comparing niche variation or niche

'discrepancy' in a radiating clade to the non-radiating sister-clade may therefore provide a test to evaluate the effect of niches on diversification rates. Furthermore, niche-shifts may require morphological or physiological change to survive and compete under novel conditions (Pearman et al. 2008), and we may therefore expect that niche evolution and trait evolution are linked. Rapid niche evolution may be more likely to occur along niche dimensions which vary at fine spatial scales (Holt 2009), and steep climatic gradients may be typical in Mediterranean systems, at least in the CFR (Linder 2005).

The history of Mediterranean-type ecosystems

The five MTEs differ in their geological and geomorphological history, topography, heterogeneity and complexity of the ecosystem, fire frequency, soil nutrient status, biotic elements and the timing of the onset of the Mediterranean climate (Thrower and Bradbury 1973, Deacon 1983, Hobbs et al. 1995, Cowling et al. 2005, Cowling et al. 2014). The near absence of recent orogenic events, subduction or glaciation during the Cenozoic have characterized the Cape and Australian MTEs as relatively stable landscapes compared to the other three MTEs (Cowling et al. 2014). Compared to Australia and South Africa, temperature and moisture oscillations associated with Pleistocene glaciations were likely more severe in North America, South America and Europe (Markgraf et al. 1995, Farmer and Cook 2013). Furthermore, the Cape and Australia have ancient basement complexes, dating back to at least the Paleozoic (Thrower and Bradbury 1973), with the exception of two episodes of uplift in southern Africa during the Miocene and Pliocene (Partridge and Maud 1987), which may have enhanced summer-aridity in the Western Cape (Tyson and Partridge 2000). Typically, these ancient landscapes and the sandy soils have led to leached out, very nutrient-poor soils in the Cape and Australia– therefore called OCBILS ('old, climatically buffered, infertile landscapes', Hopper 2009). This is in contrast to the geomorphological much more turbulent and nutrient rich land surfaces of Chile, California and the Mediterranean Basin, marked by relatively young (Late Miocene, Early Pliocene) orogenic events (Thrower and Bradbury 1973, Hopper 2009). These differences between MTEs may therefore have affected the diversification dynamics of the clades evolving in these areas.

A framework for testing the role of diversification in a spatial and phylogenetic context

In this thesis, I attempt to disentangle the complex interaction between intrinsic and extrinsic traits in the diversification of angiosperms, with the aim to better understand spatial and phylogenetic diversity patterns (Fig. 2). This approach uses a phylogenetic context to be able to reconstruct historical events (Pagel 1999), and is innovative in its attempt to combine the fields of functional traits, ecological strategies and macroevolution.

The central hypothesis of this thesis follows the idea of Crepet and Niklas (2009) that repeated innovation during a climatically dynamic period (the Cenozoic) can explain why angiosperms became species-rich and ecologically dominant in most extant ecosystems. The suggestion that this happened independently and repeatedly in several angiosperm clades, emphasizes this role of functional innovation.

Figure 2 presents a framework of themes discussed in the previous sections, and their connection to this thesis. In summary, the framework illustrates the effect of extrinsic environments and intrinsic, lineage-specific traits on radiations (arrows 5, 6 and 7 in Fig. 2), how environments shape functional traits (either by the process of adaptation [arrow 10 in Fig. 2], or by filtering lineages with suitable traits into environments [arrow 11 in Fig. 2]), and how global climate change may shape

environments by affecting their expansion and contraction (arrow 9 in Fig. 2). Furthermore, radiations create phylogenetic diversity imbalance (arrow 5 in Fig. 2) and if they are bounded to certain geographical regions, biomes or vegetation types, they can create diversity discrepancies in space (arrow 3 in Fig. 2). Two additional processes for the spatial assembly of species are recognized: time available for species assembly, and immigration (and emigration) into and out of the area (arrows 1, 2 and 3 in Fig. 2). Finally, the stability of climates and environments over time may affect the time available for the accumulation of diversity in the system (arrow 8 in Fig. 2).

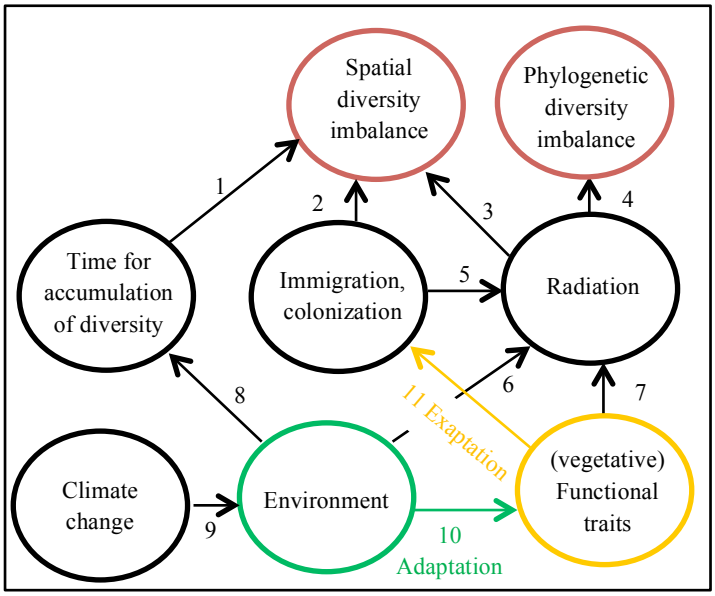

Figure 2
Framework illustrating the relationships between intrinsic (in yellow) /extrinsic (in green) variables, processes (in black) affecting and affected by these variables, and the effects of these processes on patterns of spatial and phylogenetic diversity (creating diversity imbalance, in red). Diversity in a certain area is affected by time to accumulate diversity (1), immigration of lineages (2) and evolutionary radiations (3). These radiations also create phylogenetic diversity imbalance (species-rich versus species-poor clades) (4). These radiations are affected by a number of variables and processes: colonization/immigration of new areas can provide ecological opportunities for radiation (5); environmental conditions (heterogeneity, stability) can affect diversification rates (6), as can intrinsic traits (7). Environments and their global coverage and stability over time (which could be affected by climate change, 9) affects time for accumulation of diversity (with potentially fewer extinctions in stable environments) (8). Environments may also select for certain traits ('adaptation', 10) and these traits may allow colonization of and survival in new environments ('exaptation', or pre-adaptation, 11).

Aim of this thesis

In this thesis, I will apply (parts of) this framework to several angiosperm clades which illustrate the phylogenetic, structural and ecological diversity of angiosperms worldwide, and exhibit a spatial and phylogenetic imbalance. This maximizes the number of events investigated, which will maximise the ability to make generalisations. These clades are Penaeaceae, Phyliceae, Diosmeae (chapter I), Rhamnaceae (chapters II and III), Proteaceae (chapter IV) and Poales, Fagales and Ericaceae (chapter V). The majority of the species in the clades investigated in this thesis occur in and show high endemism in Mediterranean-type ecosystems (MTEs).

In this thesis, I specifically aim to:

(1) Test the hypothesis that the interaction between (vegetative) traits and environments (climate, habitat) influences diversification rates (chapters II and IV);
(2) Apply this to lineages occurring in Mediterranean-type ecosystems to investigate the role of radiation as the cause of diversity in these systems (chapters II, III and IV);

(3) Investigate the processes of adaptation and exaptation in the evolution of functional traits during the colonization of Mediterranean-type ecosystems (chapter IV);
(4) Investigate the roles of climate evolution and trait 'disparification' during radiation (chapter V);
(5) Develop a methodology to classify intrinsic and extrinsic variables involved in radiation (chapter VI).

Chapters in this thesis

This thesis consists of seven chapters: the introduction (chapter I), five research chapters (II-VI) and concluding remarks (chapter VI). These chapters will test part of the processes illustrated in the framework in Figure 2, which I will briefly introduce below.

The Cape Floristic Region is unique in its species-richness, and even among MTEs it is exceptional (9000 species in an area of 90'000 km², ca. 70% endemic; Manning and Goldblatt 2013) (Kreft and Jetz 2007). In **chapter II** the effect of habitat colonization and trait evolution on parallel radiations in three typical Cape clades (Linder 2003) in the Cape Floristic Region is investigated (arrows 4, 5, 6 and 7 in Fig 2). These three clades are Penaeaceae Sweet ex. Guill. (Myrtales), Phyliceae Reissek ex Endl. (Rhamnaceae, Rosales) and Diosmeae DC. (Rutaceae, Sapindales). These three clades are almost entirely restricted to the CFR, colonized different vegetation types within the CFR, and exhibit a variation in functional traits, particularly in degree of leaf sclerophylly. Aridification due to climate change in the Late Miocene-Pliocene may have influenced these radiations by expansions and contraction of habitat types in the CFR (arrow 9 in Fig. 2).

Mediterranean-type ecosystems have similarities as well as differences. In **chapters III and IV** I use the Buckthorn family, Rhamnaceae Juss. (Rosales) with ca. 1055 species, to investigate the effect of these similarities and differences in the evolution of functional traits and diversification in the five Mediterranean-type ecosystems of the world (arrows 1, 2, 3 and 6 in Fig. 2 in chapter III; arrows 7, 10 and 11 in Fig. 2 in chapter IV). Rhamnaceae has a global distribution, occurs in in all five MTEs as well as in tropical rainforest biomes and deserts. The family consists of predominantly warm-temperate woody shrubs, with insect-pollinated flowers and a vegetative morphology ranging from spiny shrubs to large forest trees or lianas, and foliage ranging from aphyllous to entire, evergreen leaves, and from leaves with revolute margins to toothed deciduous leaves. Rhamnaceae has predominantly biotically-dispersed fleshy fruits or nuts. In chapter III I disentangle MTE-dependent speciation and extinction rates (arrow 3 in Fig. 2), as well as immigration rates into MTEs (arrow 2 in Fig. 2), and estimate the time of colonization of each MTE, and thus time to accumulate diversity (arrow 1 in Fig. 2). In chapter III the timing and evolution of Rhamnaceae functional traits and whether they are likely to be adaptations (arrow 10 in Fig. 2) or exaptations (arrow 11 in Fig. 2) to Mediterranean-type ecosystems is investigated. Furthermore, the effect of these traits on speciation and extinction rates in the MTEs (arrow 7 Fig. 2) is tested.

The pre-requisites for radiation may be different from the actual drivers of the radiation. In **chapter V** I investigate the role of niche and trait evolution as possible drivers of radiation in the Protea family, Proteaceae Juss. (Proteales). This Southern Hemisphere family was previously shown to have undergone evolutionary radiations in Mediterranean-type ecosystems (Sauquet et al. 2009, Reyes et al. 2015), but the drivers of these radiations remain enigmatic. Proteaceae comprises ca. 1700 species (Weston 2007) and occurs in a wide-range of habitats, such as rainforests, mountains and grasslands. This family is typical for and often dominant in the floras of the CFR and the Southwest Australian Floristic region (SWAFR). There have been many transitions between wet and dry climates (Jordan et al. 2008) and consequently there is a spectacular morphological variation within the family, from large forest trees with irregularly shaped, often lobed leaves, to small woody

shrubs with needle-like or sharply toothed leaves (Fig. 3) (Weston 2007). We estimate the rate of change in leaf functional traits, such as leaf area, specific leaf area (SLA) and leaf shape, and correlate this to the rate of niche evolution in the family, to evaluate if radiations express the highest rates (arrows 6 and 7 in Fig. 2).

Figure 3
Leaf functional trait variation in Proteaceae. a. *Grevillea leucoclada,* b. *Banksia grossa,* c. *Lomatia fraxinifolia* (juvenile), d. *Neorites kevediana* (leaflets), e. *Banksia nivea,* f. *Banksia grandis,* g. *Banksia ashbyi,* h. *Hakea victoria,* i. *Banksia victorae,* j. *Banksia victorae*, k. *Athertonia diversifolia* (juvenile), l. *Hakea pandanicarpa.*

The complex interaction between intrinsic traits and extrinsic environments during radiation challenges our understanding of the role these variables play with respect to the radiation. In **chapter VI** a classification of variables involved in radiation into backgrounds (pre-requisites), triggers and modulators during radiation, and whether they could drive radiations by being 'polymorphic' within the radiating clade, is developed (arrows 4, 5, 6 and 7 in Fig. 2). This framework allows for recognizing and classifying relevant variables in radiations and is applied to three angiosperm clades: Poales Small., Fagales Engl., and Ericaceae Juss. (Ericales). These clades illustrate the commonness of diversification rate heterogeneity in phylogenetic trees (arrow 4 in Fig. 2) and the variety of ecological traits, types and strategies among them.

In **chapter VII** I will synthesize the results of this thesis and discuss the generality of these results with respect to angiosperm diversification and functional trait innovation against a background of Cenozoic climate change. I will elucidate what processes may have affected diversification in Mediterranean-type ecosystems, and how these may affect present-day dominance of functional strategies in systems. This will provide valuable insights in angiosperm diversification and the evolution of plant functional diversity.

CHAPTER II: DIVERSIFICATION RATE SHIFTS IN THE CAPE FLORISTIC REGION: THE RIGHT TRAITS IN THE RIGHT PLACE AT THE RIGHT TIME

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Author contributions:

REO and HPL designed the research; REO, RJC, YX and HPL conducted fieldwork; REO measured most functional traits, with help from RJC and YX; REO downloaded alignments from TreeBASE and performed dating analyses; REO performed diversification rate analyses, ancestral state reconstructions and tests of correlated evolution; REO wrote the manuscript with major comments from HPL and minor comments from RJC and YX.

Abstract

Species diversity patterns are the product of diversification rate variation, but the factors influencing changes in diversification rates are poorly known. Radiation is thought to be the result of ecological opportunity: the right traits in the right environment at the right time. We test this in the Cape Floristic Region (CFR) of South Africa, in which pyrophytic heathland (fynbos) and non-pyrophytic Afromontane forest occur interdigitated. We infer transitions from forest to fynbos in three Cape clades (Penaeaceae, Phyliceae and Diosmeae) and test if they are associated with diversification rate shifts and the evolution of functional traits linked to fire, high insolation and seasonal drought. We estimate diversification rate shifts using maximum likelihood and use phylogenetic comparative methods to show that forest to fynbos shifts were associated with decreases in leaf area and specific leaf area and preceded or coincided with increases in diversification rates. Furthermore, we show that Penaeaceae, Phyliceae and Diosmeae species are typical members of their vegetation types in terms of their traits. The diversification rate shifts of Penaeaceae and Phyliceae are dated to the Miocene, when postulated aridification-driven changes in the CFR fire regimes may have triggered expansion of the fynbos at the cost of forest, providing an ecological opportunity for the diversification of fynbos lineages.

Keywords:
Afromontane forest; Cape flora; fire; fynbos; Mediterranean type ecosystem; sclerophylly; specific leaf area.

Introduction

The Cape Floristic Region (CFR) (Goldblatt 1978) of South Africa, with its unusually elevated angiosperm species diversity of ca. 9000 species in an area of 90000 km² and 68.8% endemism at the species level (Manning and Goldblatt 2013), has been the arena for some remarkable radiations (Linder 2003, Linder 2005, Linder 2008, Verboom et al. 2009). The CFR has a predominantly Mediterranean-type climate, characterized by wet winters and dry summers (Kottek et al. 2006). The species-rich, pyrophytic, nutrient-poor heathland called 'fynbos' dominates the region and co-occurs with species-poor, non-pyrophytic evergreen Afromontane forest, which is restricted to isolated fire-sheltered enclaves, often along rivers (Mucina and Geldenhuys 2006, Rebelo et al. 2006). The high species richness and endemism of fynbos may be the result of a high *in situ* diversification rate.

Theory predicts that diversification rates may be increased by 'ecological opportunity' (Simpson 1953, Baldwin and Sanderson 1998, Moore and Donoghue 2007), in combination with the appropriate traits (Arakaki et al. 2011, Drummond et al. 2012b, Near et al. 2012). Fynbos may constitute the critical ecological opportunity in the CFR. Fire, low soil nutrients, summer-drought and high insolation are typical for the fynbos vegetation (Keeley et al. 2012). These conditions limit the range of viable ecological strategies in a community, creating communities filtered for functionally similar species (Webb et al. 2002, Verdú and Pausas 2007). These conditions therefore generate a 'functional trait syndrome' by selecting for or filtering out those species that lack these traits (Ackerly 2004a), and the evolution of functional traits associated with fynbos vegetation is therefore expected to have influenced the evolutionary fate of the clades entering this system. This 'fynbos syndrome' includes highly branched slender twigs with a high density of narrow leaves with low leaf moisture content, and a shrubby growth-form, all typical for sclerophyllous, fire-spreading vegetation (van Wilgen et al. 1990, Schwilk and Ackerly 2001, Ackerly 2004b, Belcher et al. 2010). These lead to leaves with a high tissue density and low surface area per unit mass (low Specific Leaf Area, SLA). Decreases in leaf area and SLA have been shown to be related to increasing drought and nutrient

limitation (Fonseca et al. 2000, Ordoñez et al. 2009), and small leaves are correlated with high insolation (Wright et al. 2004, Cornwell and Ackerly 2009). Species in arid or seasonally-arid regions have been shown to have a low SLA (Fonseca et al. 2000, Ackerly 2004a, but see Wright et al. 2004), and evolution of evergreen sclerophyllous shrubs in fire-prone Mediterranean ecosystems in general is convergent (Mooney and Dunn 1970).

Here we use three typical Cape flora clades (Linder 2003) - Penaeaceae Sweet ex. Guill. (Myrtales), Phyliceae Reissek ex Endl. (Rhamnaceae, Rosales) and Diosmeae DC. (Rutaceae, Sapindales) - to test the hypothesis that the evolution of low SLA and small leaves is correlated with the entry into fynbos and was followed by increased rates of diversification. Penaeaceae, Phyliceae and Diosmeae are the only three Cape clades to our knowledge that are almost entirely restricted to the CFR and are represented both in the evergreen Afromontane forest and the fynbos. Furthermore, these clades are well studied phylogenetically (Richardson et al. 2001b, Richardson et al. 2004, Rutschmann 2006, Rutschmann et al. 2007, Trinder-Smith et al. 2007), and sequence data were therefore readily available. We first asked whether there were any diversification rate shifts in the three clades (**Q1**). To this end, we inferred time-calibrated phylogenetic trees and used a likelihood method to determine diversification rate shifts while correcting for incomplete taxon sampling. Then we asked whether vegetation type and leaf traits show correlated evolution (**Q2a**). We tested the null hypothesis that vegetation type and leaf trait shifts are not concordant, indicating that the evolution of low SLA and small leaves did not coincide with the transition to fynbos. We used phylogenetic comparative methods to infer ancestral states and to test for correlated evolution between vegetation type and traits. Furthermore, we tested whether forest and fynbos Penaeaceae, Phyliceae and Diosmeae are typical members of their respective vegetation types in terms of their leaf traits (**Q2b**), by comparing community trait profiles in paired forest-fynbos plots in the CFR. Finally, where we found positive answers to Q1 and Q2, we asked whether the greater diversity in fynbos compared to forest was due to an elevated diversification rate in fynbos and the evolution low SLA and small leaves (**Q3**), in other words, whether vegetation type shifts, trait shifts and diversification rate shifts were concordant between clades and in time.

Overall, we show that shifts in vegetation type and leaf traits have either preceded or coincided with the accelerated *in situ* diversification of fynbos taxa, the syndrome of low SLA and small leaves therefore being a 'precursor' for accelerated diversification. We discuss these results in the context of the complex diversification history of the CFR.

Materials and methods

Phylogenetic data

We assembled an alignment of 5123 base pairs (bp) for 25 of the 33 species of Penaeaceae and eight outgroup species of Alzateaceae and Crypteroniaceae from previously published chloroplast (*rbc*L, *ndh*F and *rpl*16-intron) and nuclear (ribosomal 18S and 26S) DNA sequence data (TreeBASE study number S1802) (Schönenberger and Conti 2003, Rutschmann et al. 2007). The Phyliceae dataset consisted of previously published *trn*L-F and internal transcribed spacer (ITS) sequence data (Richardson et al. 2001b) which we expanded with additional sequence data (GenBank accession numbers are provided in Table S1). DNA extraction, DNA sequencing and sequence aligning procedures were performed as described in Richardson *et al.* (2001b). The final dataset included 47 of the 136 species of Phyliceae augmented with 10 outgroup species from other Rhamnaceae genera in the ziziphoid group, and the alignment consisted of 1847 bp. The aligned Diosmeae dataset consisted of 2522 bp of previously published plastid sequence data (*trn*H-*psb*A intergenic spacer, *atp*B-*rbc*L intergenic spacer and *rpl*16-intron) (TreeBASE study number S1814) (Trinder-Smith et al. 2007). All

currently recognized genera of Diosmeae, each represented by between one and five species, were included in this study, resulting in a total of 26 of the 276 species of Diosmeae, and four outgroup species of genera in Rutaceae.

Timing of divergences

The joint posterior distribution of topologies and divergence times were estimated for all three clades using Bayesian MCMC implemented in BEAST 1.7.1 (Drummond and Rambaut 2007) under a lognormal relaxed clock model and a pure-birth speciation process. The GTR + Γ nucleotide substitution model was used for all loci, and genomic regions were unlinked to accommodate differences in mean substitution rates between chloroplast and nuclear DNA. Priors for the mean substitution rates and the calibrated Yule (Heled and Drummond 2011) rate were estimated in a test run in which the prior of the calibrated node was constrained for its mean age. A final run was performed using the estimated substitution rates of the test run as prior distributions for these rates, and relaxing the calibration prior to allow for uncertainty in the timing of divergences. We explored the use of normal and lognormal calibration prior distributions in Penaeaceae, Phyliceae and Diosmeae, but as age estimates did not differ much between both approaches, we will only present the results based on the normally distributed priors here (for BEAST settings and comparison of divergence time estimates under normal and lognormal priors see Table S2). We performed two MCMC runs of 20 (Penaeaceae, Diosmeae) or 30 (Phyliceae) million generations, sampling every 1000 generations. The first 10% generations were discarded as burn-in. Convergence of the model parameters of the MCMC chains was checked in Tracer 1.5 (Rambaut and Drummond 2007), and topological convergence using the online service AWTY (Nylander et al. 2008).

Rutschmann *et al.* (2007) calibrated the Penaeaceae and related families using six fossils and several calibration sets. They estimated the crown age of the clade containing Crypteroniaceae, Alzataceae and Penaeaceae to be between 72.8 – 81.5 Ma. We set the prior distribution of the age of this group as a normal distribution with a mean of 77.15 and a standard deviation of 2.7 Ma, and constrained it to be monophyletic. Speciation was set to a calibrated Yule prior with a lognormal distribution with a log (mean) of 0.11 and log (standard deviation) of 0.27.

Richardson *et al.* (2004) calibrated the Rhamnaceae using the mean stem age estimates of Wikström *et al.* (2001) of 62 and 64 Ma, resulting in an age estimate of 22.9 –23.6 ± 3.1 Ma for Phyliceae. We followed this calibration, and set a normal distribution with a mean of 23.3 and a standard deviation of 2.1 Ma. The calibrated Yule prior containing the monophyletic Phyliceae was set with a lognormal distribution with log (mean) of 0.056 and a log (standard deviation) of 0.38. We acknowledge that a tertiary calibration may not be reliable for estimating divergence times. Although fossils within the Phyliceae are lacking, there are fossils associated with the genera *Ceanothus* and *Colubrina*, which we included as outgroup lineages. We tested the reliability of the tertiary calibration by performing an analysis in which we calibrated the stem nodes of *Ceanothus* (uniform prior, 18 – 96) and *Colubrina* (uniform prior, 28.4 – 96) based on, respectively, a fossil of *Ceanothus precuneatus* from Middlegate (USA) (Axelrod 1985) and a fossil of *Colubrina spireaefolia* from Florrisant (USA) (Manchester 2001) and comparing the obtained highest posterior density (HPD) of the Phyliceae crown node age of this analysis to the HPD obtained from the analysis using a tertiary calibration. The minimum age of the calibration prior was determined by the age of the fossils; the maximum age was taken from the 'Rose Creek Flower' fossil, which shows morphological affinities in floral type with Rosales (Rhamnaceae), as well as with Saxifragales (Basinger and Dilcher 1984). It may therefore be associated with an ancestral lineage older than the ancestor of the extant Rhamnaceae, thereby providing a realistic maximum age constraint. The HPD of the Phyliceae crown node age as estimated with the tertiary calibration fell well within the HPD of the Phyliceae crown node age as estimated by means of two fossil calibrations in the outgroup lineages (Table S3).

Therefore, we have confidence in the estimated divergence times obtained from the analysis using a tertiary calibration, and all subsequent analyses were performed on phylogenetic trees obtained from this analysis.

Diosmeae were calibrated based on the crown age estimate of Diosmeae, *Boronia* and *Zanthoxylum* (95% HPD 45 – 69.5 Ma) of Rutaceae by Salvo *et al.* (2010). This was based on a broadly sampled family phylogeny and four fossil calibrations. The phylogenetic analysis of Rutaceae based on chloroplast DNA by Groppo et al. (2008) indicated that *Vepris* is nested within this clade. We therefore used the HPD as estimated by Salvo *et al.* (2010) as a secondary calibration for the clade including Diosmeae, *Boronia*, *Zanthoxylum* and *Vepris*, and the prior followed a normal distribution with a mean of 57 and a standard deviation of 7.5 Ma. The calibrated Yule prior was set with a lognormal distribution with a log (mean) of 0.008 and a log (standard deviation) of 1.

Diversification rate estimates

To test for diversification rate shifts (**Q1**), we conducted diversification rate analyses on the species trees and genus trees by pruning the outgroup lineages and all but one tip per species or genus respectively from the maximum clade credibility (MCC) phylogenetic trees and from 100 post-burn-in trees from the BEAST analysis. The multiMEDUSA function (Alfaro et al. 2009), available in the Geiger library of R (Harmon et al. 2008), was used to locate significant accelerations and slowdowns in the diversification rate, without *a priori* assumptions of the phylogenetic location of the shift. MEDUSA uses a likelihood approach to select the optimal speciation (λ) and extinction (μ) parameters to account for the current pattern of diversity in a clade, thus considering branch-length distribution as well as species counts. Unsampled taxa may be assigned to the tips of the phylogenetic tree to account for incomplete taxon sampling. MEDUSA seeks the smallest number of shifts at which changes in λ and/or μ occur, at which the addition of a further shift does not result in a significantly better explanation, given the data, based on model selection using AIC. The AIC threshold value which ensures a significant rate shift ($P < 0.05$) is calculated automatically by MEDUSA based on the number of tips in the tree. For each shift, MEDUSA selects the best model (pure-birth or birth-death), and will indicate whether the shift occurred at the stem node or the crown node of a clade.

To be able to assign species richness numbers to the tips, we compiled taxonomic data from recent publications (Schönenberger and Conti 2003, Trinder-Smith et al. 2007, Sebola and Balkwill 2009, Hämmerli unpublished). Unfortunately, confidently assigning unsampled species to tips often results in dropping species trees to genus trees, because the closest relatives of the unsampled species may be unknown, but can be assumed to be in the same genus as the sampled species of the genus. Dropping tips means fewer splitting events to infer diversification rate shifts over, resulting in less precise identification of the timing and topological position of the shift. We accommodated this problem by running MEDUSA multiple times using different approaches (Table S4 summarizes the advantages and disadvantages of each approach), and comparing the results and stability of the shifts. First, we ignored unsampled taxa, thereby assuming that species sampling was even for the study group. Second, we assigned species richness of an under-sampled genus equally to the species (tips) representing this genus in the phylogeny, thus assuming that sampling was even within the genus. For example, *Stylapterus* (Penaeaceae) has eight species, but only four species were present in the phylogeny. Each of these four tips was therefore assigned a species richness value of two (Table S5). This method allows a more thorough identification of the actual location of the diversification rate shift by retaining all splitting events. Third, we dropped tips to genus-level, and assigned all species known for the genus to this tip, in case the genus was shown to be monophyletic. In case of non-monophyletic genera, we did two things: we assigned the species number to the (well supported) common ancestor of the genus, which also includes other species or genera, and in addition we did multiple runs in which we considered all possible combinations of the topological placement of the

genus (in each run another tip received all unsampled species of the genus), and investigated whether this affected the location of the diversification rate shift. Finally, we simply dropped tips to retain a forest and a fynbos tip, for direct comparison of differences in diversification rates between forest and fynbos. By including two forest or fynbos 'ingroup' tips, we were able to distinguish between a stem node and a crown node shift (these tips included the first diverging lineage and another randomly chosen lineage of the forest or fynbos clade, and both tips received half of the species known to occur in the clade).

Vegetation type and traits

We established which trait attributes are typical of forest or fynbos by comparing their community trait profiles in a paired block design. Ten geographically separated sites in the south-western and southern Cape were each sampled with a set of paired forest-fynbos plots. In each plot five transects of 50 x 1 m were placed in random directions (Table S6). Leaf area (mm^2) and SLA (leaf area divided by its oven dried leaf mass, mm^2/mg) of on average ~70% of the angiosperm species along the transect was scored, following the protocols of Cornelissen *et al.* (2003). The ~30% missing species were due to a lack of material when too few mature individuals were encountered in the field. For broad-leaved forest species the mean leaf area and SLA per species were calculated based on a leaf from each of 10 individuals, for small ericoid leaves we took 10 – 50 leaves per individual and treated these as one leaf, to reduce the size- and weight-error associated with small and light leaves. We repeated this for five individuals. In order to test whether forest and fynbos Penaeaceae, Phyliceae and Diosmeae are typical members of their respective vegetation types in terms of their functional traits (**Q2b**), we also scored SLA and leaf area for Penaeaceae, Phyliceae and Diosmeae species. Leaf area and vegetation type data for the species of Penaeaceae, Alzataceae and Crypteroniaceae were obtained from Rutschmann (2006), and for leaf area and SLA for Phyliceae from Ackerly (2004a). In addition, we sampled traits from Penaeaceae, Phyliceae and Diosmeae species in the field, and for the remaining species, which were not encountered in the field but included in the phylogeny, we used herbarium specimens for leaf measurements, if these were available, adjusting the size by 12% to correct for leaf shrinkage of broad-leaved forest species (Maharjan et al. 2011).

Leaf area and SLA species means were log transformed to normalize the data. The effect of vegetation type and site on the variance in log (SLA) and log (area) was investigated with a two-way ANOVA. To verify the morphological association of forest and fynbos Penaeaceae, Phyliceae and Diosmeae to the corresponding vegetation type, log (area) was plotted against log (SLA) for all species, which allowed us to calculate the Euclidean distance between all Penaeaceae, Phyliceae and Diosmeae species to the fynbos and forest vegetation members. To assess the significance of this distance, we generated 999 randomized vegetation type matrices, in which vegetation type was randomly shuffled among the species, and again calculated the distance of Penaeaceae, Phyliceae and Diosmeae species to their corresponding vegetation type members. If our observed distance was detected less than 5% at random ($P < 0.05$), we considered it significant.

Ancestral vegetation type and vegetation type/trait correlations

To explore evolutionary shifts and correlations in vegetation type and traits in the three clades, vegetation type, leaf area and SLA for Penaeaceae, Phyliceae and Diosmeae species were assigned to the corresponding taxa at the tips of the phylogenetic trees and analysed using Bayesian methods. For *Olinia* in Penaeaceae we could only collect trait data for two out of nine species, of which only one was also present in the phylogeny. We therefore assigned trait data of *Olinia huillensis* subs. *discolor*, for which we collected trait data but which was not included in the phylogeny, to *O. vanguerioides*, which was included in the phylogeny. *O. huillensis* subs. *discolor* belongs to the *O. rochetiana sensu lato* complex and is morphologically and geographically closest to *O. vanguerioides* (Sebola and

Balkwill 2009).

Ancestral state reconstructions were performed on 100 randomly selected post-burn-in trees from the BEAST analyses in order to account for the effects of variation in estimated tree topology and branch lengths on the ancestral states. We sampled the posterior distribution of ancestral vegetation type states in BAYESTRAITS 1.0 (Pagel and Meade 2006). We selected the node of interest by defining the descendants of that node, regardless of whether they form a clade in all tree topologies. By using the *fossil* command, we compared a model in which the harmonic mean likelihood is calculated when the node is forced to have the forest state, versus a model in which we force it to have the fynbos state. We used a reversible jump (RJ)-MCMC model with priors obtained from the hyperprior approach and used an exponential prior seeded from a uniform on the interval of 0 – 10 or 0 – 30. Runs and prior intervals were optimised during preliminary runs to establish a 'ratedev' value which resulted in acceptance rates between 0.2 and 0.4. All analyses were run for 10^7 generations, and replicated five times. The burn-in was 10% (10^6 generations) and models and rate parameters were sampled every 100th iteration (BAYESTRAITS settings are shown in Table S7). Difference between the models was assessed by Bayes Factors (BFs), in which log BF = $2(P(D\|M_1) - P(D\|M_0))$, and $P(D\|M)$ is approximated by the harmonic mean of likelihoods averaged from the five runs. The model with the highest harmonic mean of likelihoods is regarded as the better one, and a log BF of 2 – 5 is interpreted as positive, 5 – 10 as strong and > 10 as highly significant (Pagel and Meade 2006).

To test the hypothesis for correlated evolution between vegetation type and leaf traits (**Q2a**), we used the same settings as used for the estimations of the ancestral vegetation types in BAYESTRAITS, except for the number of generations (5 x 10^6) and the burn-in (5 x 10^5 generations). The data were made binary by categorizing SLA into ≤ 5 mm²/mg and > 5 mm²/mg following Meers et al. (2008) and leaf area into leptophyllous/nanophyllous leaves (0 – 224 mm²) and microphyllous/mesophyllous leaves (225 – 18225 mm²) following Ellis et al. (2009). The categories low SLA and small leaves correspond to the lower end of the distribution of these traits found in higher plants (Cornwell et al. 2014). We compared the log BFs of independent (i.e. uncorrelated) and dependent (i.e. correlated) models using the function DISCRETE to indicate (1) whether SLA and leaf area are correlated and (2) whether SLA and/or leaf area are correlated to vegetation type.

Vegetation type and diversification rate

To test the hypothesis that the greater diversity in fynbos compared to forest was due to an elevated diversification rate in fynbos (**Q3**), we used the BiSSE algorithm (Binary State Speciation and Extinction) (Maddison et al. 2007, FitzJohn et al. 2009). However, it has been shown that BiSSE performs poorly on phylogenies with fewer than 300 tips or a tip-ratio in which fewer than 10% of the species is in one of the two states, due to low power (Davis et al. 2013). Our phylogenetic trees of Penaeaceae, Phyliceae and Diosmeae contain 33, 47 and 26 species, and have a tip ratio of 14/19 (58% of forest and 83% of fynbos species sampled), 2/45 (100% of forest and 34% of fynbos species sampled) and 1/25 (100 % of forest and 9% of fynbos species sampled) for the forest/fynbos states respectively. We therefore estimated diversification rate shifts and habitat shifts independently, but see Appendix A and Figure S1 for exploration of the BiSSE algorithm for Penaeaceae, Phyliceae and Diosmeae.

Results

Diversification rate shifts

The backbones of the phylogenies for Penaeaceae, Phyliceae and Diosmeae are well resolved with strong support as indicated by posterior probabilities (p.p. > 0.85) (Figure S2 and S3), and are congruent with previously published phylogenies (Richardson et al. 2001b, Rutschmann et al. 2007, Trinder-Smith et al. 2007). However, contrasting previous results by Trinder-Smith et al. (2007) who found support for the monophyletic Diosmeae (p.p. = 0.92), we detected weak support (p.p. = 0.7) for the inclusion of the Australian *Boronia* nested within the Diosmeae clade (Figure S2). We found support for at least one diversification rate shift in each of the three clades, thus rejecting the null hypothesis of constant diversification rates (**Q1**, Figure 1, Table 1a). The direction (acceleration or slowdown) and timing of the shifts varied between approaches and phylogenetic trees. The most reliable and consistent results are discussed here (see Appendix B for arguments why) and results for all approaches are shown in Table S8. Table S9 shows AIC values for each run.

For Penaeaceae, a rate shift was detected in 98% of the trees. Against the background rate, a Late Miocene increase in diversification rate was found at the Penaeaeae crown (i.e. tribe Penaeaeae are the fynbos members within Penaeaceae) in 68% of the trees (Figure 1, Table 1a). In the remaining 30% of the trees, the rate shift was detected within the Penaeaeae, two or three nodes more recently (mostly excluding *Endonema* and *Glischrocolla*, but due to topological uncertainty sometimes other species were excluded). The forest/fynbos sister clade comparison indicated a significant (but moderate) slowdown in the forest lineage (Table 1b), but including the crown of the fynbos lineages recovered the increase in rate at the crown of the Penaeaeae in all 100 trees.

For Phyliceae, a rate shift was detected in 99% of the trees. An Oligocene/Miocene increase in the diversification rate at the stem node of *Phylica* was detected in 68% of the trees (Figure 1, Table 1a) and in 10% of the trees a slowdown in the sister clade of *Phylica* was detected. In the remaining 21% of the trees, *Phylica* was sister to *Noltea* and these were sister to *Nesiota* and *Trichocephalus*, for which a slowdown in the *Nesiota*/*Trichocephalus* clade was inferred. The forest/fynbos sister clade comparison indicated a significant slowdown in the forest clade (Table 1b), but including the crown of *Phylica* recovered the increase in diversification rate at the *Phylica* stem node in 78% of the trees, and in 6% of the trees the shift occurred at the *Phylica* crown node.

For Diosmeae, a rate shift was detected in 100% of the trees. Against the background rate, an increase in the net diversification rate at the crown node of Diosmeae excluding *Calodendrum capense* in the Oligocene/ Miocene was inferred in 40% of the trees (Figure 1, Table 1a), and a rate slowdown in the lineage subtending *C. capense* in 60% of the trees. This same slowdown was detected in the forest/fynbos sister clade comparison in 100% of the trees (Table 1b), but when including the crown of the fynbos lineages, the shift disappeared, and no significant increase or decrease in the diversification rate was detected.

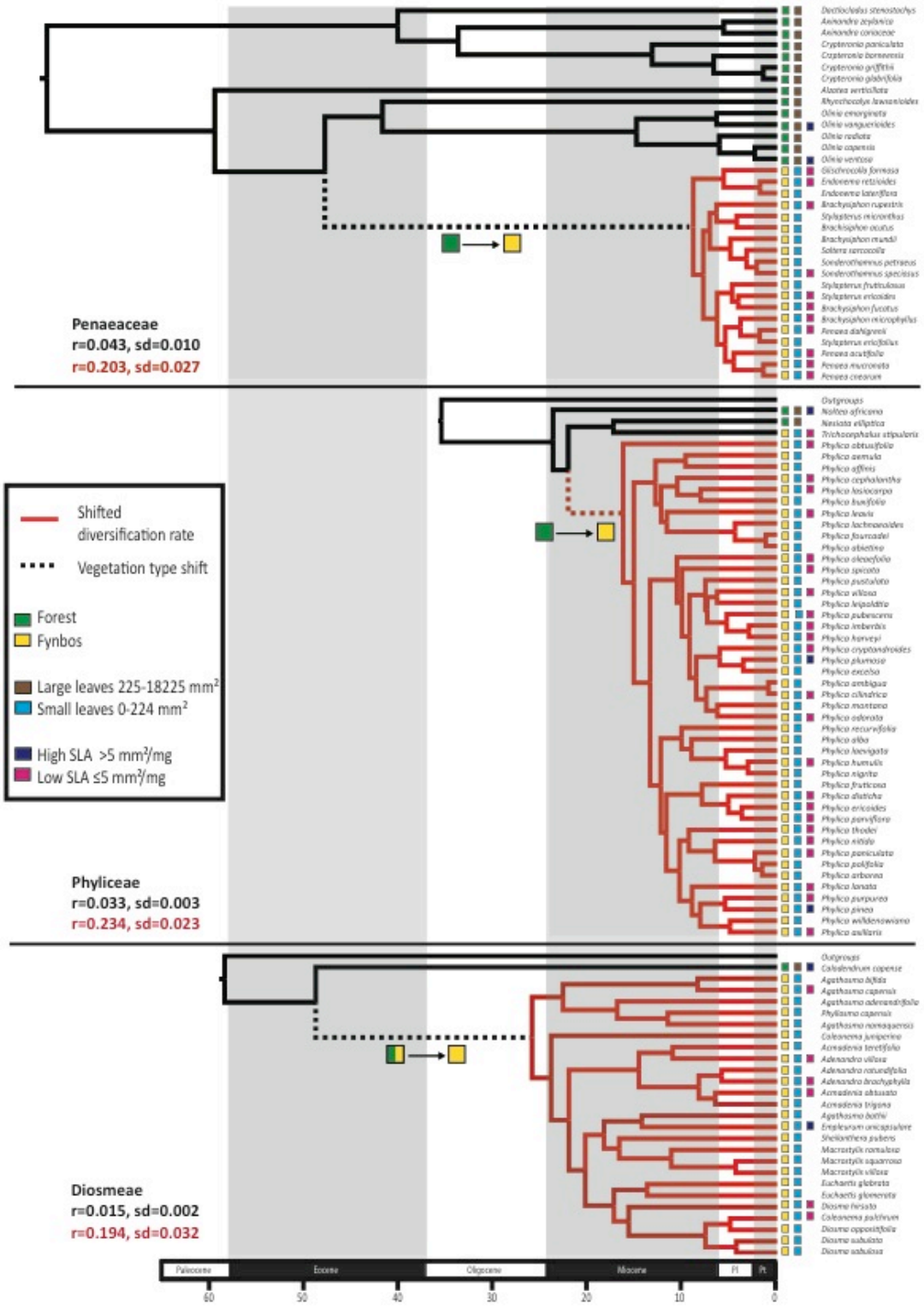

Figure 1

Diversification rate shifts, vegetation type shifts and associated traits (leaf area and SLA) in Penaeaceae, Phyliceae and Diosmeae as inferred by MEDUSA and BAYESTRAITS. The mean net diversification rate r (lineages/Myr) and the standard deviation (sd) as estimated over the % of 100 BEAST trees which showed a significant shift is indicated for the lineages which had a significant shift in the diversification rate (colour: red), and for the non-shifted lineages (colour: black) within each clade.

Vegetation type shifts and vegetation type/trait correlations

The null hypothesis that changes in leaf area and SLA were independent of the transition between vegetation types was rejected (**Q2a**). Significant shifts from forest to fynbos were inferred for Penaeaceae and Phyliceae (Table 1b, Table 2a, Figure 1), indicated by Bayes Factor support for the ancestral reconstruction of one vegetation type over the other for a particular node, and the subsequent change of vegetation type in the descending node(s). Furthermore, a significant evolutionary correlation between vegetation types and traits was inferred for all three clades (Table 2b). Forest-to-fynbos shifts were associated with a shift from large microphyllous/mesophyllous leaves to smaller leptophyllous/nanophyllous leaves, and from a high (> 5 mm²/mg) to a low (≤ 5 mm²/mg) SLA (Figure 1).

In Penaeaceae, the transition from forest to fynbos was inferred along the branch leading to the crown node of the Penaeaeae; in Phyliceae, this shift was inferred along the branch leading from the *Phylica* stem node to the *Phylica* crown node; and in Diosmeae the ancestor of the Diosmeae excluding *Calodendrum capense* was inferred to occur in fynbos. However, as the significance of the forest or fynbos state at the diosmeoid ancestor could not statistically be detected, due to the single forest lineage (*C. capense*) in the Diosmeae clade, the exact topological position of the transition between vegetation types could not be inferred.

Vegetation type trait profile

Leaf area and SLA were measured for a total of 97 forest and 121 fynbos species (data available from the TRY database: www.try-db.org). The variation in leaf area and SLA was correlated to vegetation type (leaf area: $F = 165.80$, $P < 0.0001$; SLA: $F = 89.15$, $P < 0.0001$) and not to site (leaf area: $F = 1.63$, $P = 0.104$; SLA: $F = 1.61$, $P = 0.11$) or to the interaction between vegetation type and site (leaf area: $F = 1.85$, $P = 0.059$; SLA: $F = 1.10$, $P = 0.36$). The distribution of distances of forest or fynbos Penaeaceae, Phyliceae and Diosmeae to forest or fynbos vegetation type members respectively after randomizing vegetation type, indicated that the observed distance to both was significantly shorter ($P < 0.05$) (i.e. higher similarity) than expected by chance, supporting the hypothesis that forest and fynbos Penaeaceae, Phyliceae and Diosmeae can be regarded as typical members of their vegetation types (**Q2b**). The only exception is *Empleurum unicapsulare* in the Diosmeae, which appeared closer to forest than to fynbos for leaf area and SLA (Figure 2).

Table 1

a) Mean net diversification rate (r in lineages/Myr) and standard deviation (sd) and timing (Epoch and 95% HPD Ma) of diversification rate shifts when compared to the root (non-shifted, background) rate in Penaeaceae, Phyliceae and Diosmeae as estimated by MEDUSA. These results indicate a ~5, ~7 and ~13 fold diversification rate increase in Penaeaceae, Phyliceae and Diosmeae respectively. b) Mean net diversification rate (r in lineages/Myr) and standard deviation (sd) of forest and fynbos lineages in Penaeaceae, Phyliceae and Diosmeae, based on the forest/fynbos sister group comparison with MEDUSA. The timing of the vegetation type shifts (Epoch and 95% HPD Ma) as estimated with BAYESTRAITS is also indicated.

a)

Clade	**Diversification rate (mean r and sd over % of trees which showed a significant shift)**	**Location of rate shift (node)**	**Direction of rate shift (↑=increase, ↓=decrease)**	**Timing of rate shift (Epoch)**	**Timing of rate shift (Ma)**
Penaeaceae	Root: mean=0.043; sd=0.01 Rate shift: mean=0.203; sd=0.027	Penaeaeae crown	↑	Late Miocene	5.73 – 11.48
Phyliceae	Root: mean=0.033; sd=0.003 Rate shift: mean=0.234; sd=0.023	*Phylica* stem	↑	Oligocene-Miocene	12.46 – 25.01
Diosmeae	Root: mean=0.015; sd=0.002 Rate shift: mean=0.194; sd=0.032	Diosmeae excluding *Calodendrum* crown	↑	Oligocene-Miocene	16.41 – 34.88

b)

Clade	**Diversification rates in vegetation types (mean r and sd over % of trees which showed a significant shift)**	**Location of vegetation type shift (branch)**	**Direction of vegetation type shift (→ = to)**	**Timing of vegetation type shift (Epoch)**	**Timing of vegetation type shift (Ma)**
Penaeaceae	Forest: mean=0.05; sd=0.007 Fynbos: mean=0.069; sd=0.01	Penaeaeae stem to crown	Forest → Fynbos	Paleocene-Late Miocene	5.73 – 58.6
Phyliceae	Forest: mean=0.048; sd=0.004 Fynbos: mean=0.214; sd=0.019	*Phylica* stem to crown	Forest → Fynbos	Oligocene-Middle Miocene	12.46 – 25.01
Diosmeae	Forest: mean=~0; sd=~0 Fynbos: mean=0.139; sd=0.035	Diosmeae excluding *Calodendrum* stem to crown	Forest/Fynbos → Fynbos	Paleocene-Middle Miocene	16.41 – 62.90

Table 2

BAYESTRAITS results. a) Ancestral habitat reconstruction, comparing BFs of a model with constrained forest versus fynbos state at specified nodes. Log BF value: 2 – 5 positive, 5 – 10 strong evidence, > 10 very strong evidence. b) Trait-habitat correlations, comparing Bayes Factors (BFs) of dependent (correlated) and independent (uncorrelated) models.

a)

Clade	***fossil* Node**	**Forest**	**Fynbos**	**log BF**	**Conclusion**
		State 0: $P(D\|M)$	State 1: $P(D\|M)$	$2(P(D\|M_I)-P(D\|M_D))$	
Penaeaceae	Penaeaeae stem	-6.76	-7.81	2.10	Forest
	Penaeaeae crown	-15.61	-6.76	17.70	Fynbos
Phyliceae	Phyliceae crown	-5.90	-8.63	5.46	Forest
	Phylica stem	-6.60	-8.67	4.14	Forest
	Phylica crown	-10.00	-6.64	6.73	Fynbos
Diosmeae	Diosmeae except *Calodendrum* stem	-7.90	-8.20	0.60	None
	Diosmeae except *Calodendrum* crown	-9.85	-7.59	4.52	Fynbos

b)

Clade	**Correlation**	**Independent**	**Dependent**	**log BF**	**Conclusion**
		$P(D\|M)$	$P(D\|M)$	$2(P(D\|M_I)-P(D\|M_D))$	
Penaeaceae	Habitat-Leaf area	-10.21	-6.08	8.26	Dependent
	Habitat-SLA	-16.98	-13.68	6.61	Dependent
	Leaf area-SLA	-16.98	-13.68	6.61	Dependent
Phyliceae	Habitat-Leaf area	-12.43	-6.34	12.17	Dependent
	Habitat-SLA	-19.80	-16.68	6.23	Dependent
	Leaf area-SLA	-15.49	-13.08	4.83	Dependent
Diosmeae	Habitat-Leaf area	-11.88	-9.79	4.17	Dependent
	Habitat-SLA	-12.17	-9.35	5.66	Dependent
	Leaf area-SLA	-14.64	-10.05	9.18	Dependent

Vegetation type and diversification rate

The BiSSE results (Appendix A, Figure S1) are concordant with our findings here, indicating higher diversification rates of fynbos lineages compared to forest lineages for Penaeaceae and Phyliceae, although only for Penaeaceae the results show statistical significance (**Q3**). However, as indicated by Davis et al. (2013), a BiSSE run with low power can fail in rejecting the null hypothesis of equal rates between states when the alternative hypothesis is true (Type II error). This may be the reason for the non-significant results for Phyliceae and Diosmeae.

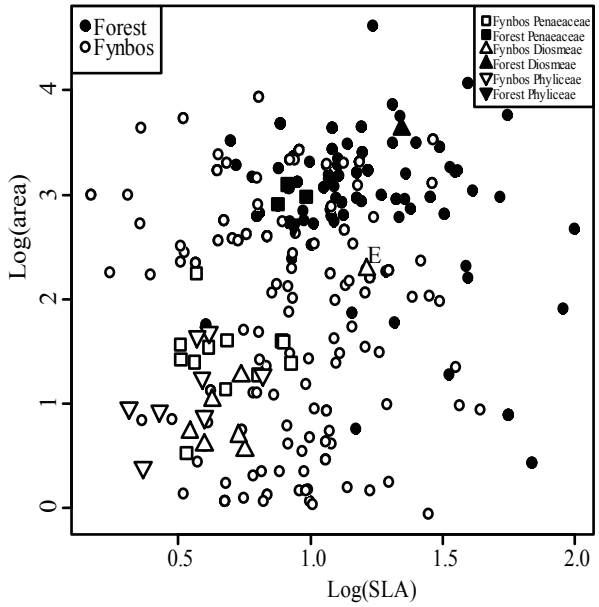

Figure 2
Forest and fynbos species of the pooled dataset plotted as log (area) against log (SLA). Forest Penaeaceae, Phyliceae and Diosmeae are typical for their host vegetation type communities based on these traits, as are fynbos Penaeaceae, Phyliceae and Diosmeae, except from *Empleurum unicapsulare*, indicated with **E**.

Discussion

There is concordance between diversification rate shifts, vegetation type shifts and trait shifts in Penaeaceae, Phyliceae and Diosmeae (**Q3**). The inferred topological positions of the shifts in diversification rates, vegetation type and traits occur on the same (Phyliceae, Diosmeae in 60% of the trees) or adjacent internodes (Penaeaceae, Diosmeae in 40% of the trees) (Figure 1). Fynbos lineages typically have smaller leaves and lower SLA than forest lineages, and in all three investigated clades (Penaeaceae, Phyliceae and Diosmeae) shifts to fynbos were correlated with the evolution of smaller leaves and lower SLA. The correlation between leaf area and SLA suggests that these two traits may be genetically (Rebetzke et al. 2004, Donovan et al. 2011) and/or physiologically linked (Westoby et al. 2002) and can be considered a (functional) trait syndrome associated with the forest/fynbos dichotomy. Furthermore, Penaeaceae, Phyliceae and Diosmeae species are typical members of their forest or fynbos vegetation types. Our results suggest that increased rates of diversification may be the consequence of a match of traits to ecological opportunity. The direction (acceleration or slowdown) and timing of the diversification rate shifts varied between diversification rate approaches, phylogenetic trees and between Penaeaceae, Phyliceae and Diosmeae, but mostly indicated a Late Miocene rate increase in Penaeaceae, an Oligocene/Miocene rate increase in Phyliceae and a Paleocene (in 60% of the trees) or Oligocene/Miocene (in 40% of the trees) rate increase in Diosmeae. Timing of the entry into fynbos shows overlap between the clades, but could have happened anytime between the Paleocene (stem node) and Late Miocene (crown node) for Penaeaceae, between the Paleocene (stem node) and Oligocene/ Miocene (crown node) for Diosmeae, and between the Oligocene (stem node) and Miocene (crown node) for Phyliceae.

The differences in species richness between forests and fynbos in the CFR may be the result

of three historical processes: recruitment via colonization events, differences in time for *in situ* diversification, or different *in situ* diversification rates. The Cape flora is not unusual in its ability to recruit lineages from all continents (Linder 2005) and recruitment may be on-going (Galley and Linder 2006). However, higher species diversity in the fynbos is unlikely to be explained by recruitment differences between forest and fynbos in the CFR, because the number of families (as a proxy for colonization events) encountered in forest and fynbos was similar in this study (Figure S4). The time for diversification and thus the age of a lineage could also influence the species richness in an area (McPeek and Brown 2007). However, transitions happened from an ancestral forest state (Penaeaceae, Phyliceae) to a derived fynbos state, or the forests and fynbos states are sister states (Diosmeae). This suggests that these lineages either first diversified in forest before occupying fynbos, or at the same time, and time can therefore not explain the higher species richness of fynbos compared to forest. The third explanation, a higher *in situ* diversification rate, is corroborated by our results. The only previous comparison of *in situ* diversification between vegetation types in the CFR has been for the grass genus *Ehrharta,* which radiated when shifting from fynbos to succulent karoo environments (Verboom et al. 2003). Other comparisons have been for clades in the Cape and adjacent Afromontane regions. The diversification rate of *Protea* is the same within the CFR as in the uplands of tropical Africa (Valente et al. 2010a). This is not matched by the Proteaceae as a whole, which have higher diversification rates in the Mediterranean ecosystem hotspots, including the CFR, than in other areas of their total distribution (Sauquet et al. 2009). Galley *et al.* (2007) infer dispersals in Cape lineages (*Disa*, Irideae, *Pentaschistis* and Restionaceae) from the Cape to tropical Africa, but the amount of *in situ* diversification is different between areas and clades. We add to the understanding of *in situ* diversification in the CFR, by showing that differences in the diversity among vegetation types within the CFR can be explained by different rates of net *in situ* diversification.

Nevertheless, the mechanism behind the higher *in situ* diversification rates of fynbos lineages compared to forest lineages remains unclear, and may have resulted from higher speciation rates, lower extinction rates, or both. Goldberg *et al.* (2011) found increased speciation rates but unchanged extinction rates in heathy, sclerophyllous, species-rich chaparral, compared to forested regions in California. This suggests that the generally high species richness of Mediterranean-type vegetation (Cowling et al. 1996) could be the result of an elevated speciation rate. Alternatively, fire, which has become an important element in the CFR at least since the Middle Miocene (Bytebier et al. 2010), may have extended the heathlands at the cost of the afromontane forests (Coetzee and Rogers 1982), and this contraction and fragmentation of forest vegetation may have increased extinction rates of forest lineages. Concordantly, newly available fynbos habitat space may have provided 'ecological opportunities' for speciation.

Furthermore, higher diversification rates in fynbos shrubs compared to forest trees is consistent with arguments that generation time may influence diversification rates, and in particular, trees generally have lower diversification rates than shrubs (Verdú 2002). This could be exacerbated by the effects of fire. Many fynbos shrubs are killed by fire, and populations are re-established from seeds. These obligate reseeder species have short, non-overlapping generations, and this may result in higher diversification rates (Wells 1969, Thomas et al. 2010). Although tests of the differential diversification of resprouters versus seeders have not found significant results (Verdú et al. 2007), at a microevolutionary level Segarra-Moragues and Ojeda (2010) found an almost double genetic differentiation between seeder populations compared to resprouter-populations in the dimorphic fynbos shrub *Erica coccinea*.

The ancestral vegetation type reconstructions indicate a Miocene crown age for the fynbos ancestral state and the onset of the diversification of the fynbos Penaeaceae, Phyliceae and Diosmeae (although the 95% HPD suggests an Oligocene/Miocene crown age for the fynbos Diosmeae). Verboom et al. (2009) suggested that most fynbos lineages are younger than 15 My (Middle

Miocene), which can be regarded as the start of the 'modern' planet (Potter and Szatmari 2009). This may coincide with the start of the modern, pyrophytic, summer-dry Cape ecosystem (Levyns 1964, Bytebier et al. 2010). This unusual seasonal climate may have resulted from the increased Antarctic glaciation lowering the South Atlantic sea surface temperatures (Zachos et al. 2001), thus increasing the strength of the high pressure cell and blocking the penetration of Indian Ocean moisture across the subcontinent (Diekmann et al. 2003, Dupont et al. 2011). However, several typical Cape clades (Restionaceae, Proteaceae, and possibly also the fynbos Diosmeae) date to the Oligocene (Linder and Hardy 2004, Sauquet et al. 2009), and thus predate this 'modern world'. Furthermore, the transition from forest to fynbos and correlated trait shifts could have happened anytime between the Paleocene and the Miocene (Penaeaceae, Diosmeae) or the Oligocene and the Miocene (Phyliceae). This implies that heathy fynbos in the CFR may be much older, and lineages could have evolved low SLA and small leaves in response to oligotrophic habitats, before the onset of the summer-dry Cape ecosystem (Keeley et al. 2012). The same has been suggested for the evolution of scleromorphic traits in humid-climate Late Paleocene Australian Proteaceae, pre-adapting these taxa to xeric conditions (Hill 1998).

Conclusion

We show that diversification of the Cape flora is affected by differences in diversification rates between interdigitated vegetation types. Contraction and expansion of these vegetation types due to Middle Miocene climate change and fire frequency may have affected the establishment and loss of habitats. The establishment of new habitats, analogous to the seasonally arid condition for cacti (Arakaki et al. 2011) or savanna conditions for grasslands (Beerling and Osborne 2006), may have facilitated speciation of the fynbos flora. Alternatively, or additionally, the loss of afromontane forest habitats may have caused extinction of forest lineages. Fynbos conditions, such as oligotrophic soils, seasonal-drought and fire, may have selected for low SLA and small leaves. This suggests that the remarkable richness of the Cape flora could be the result of the match between ecological opportunity and the appropriate trait syndrome.

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Supporting Information Chapter II

Appendix A

BiSSE (Binary State Speciation and Extinction) methods and results

We used the BiSSE (Binary State Speciation and Extinction) algorithm (Maddison et al. 2007) to test if speciation and extinction vary significantly between forest and fynbos lineages. Importantly, the extended version of BiSSE in the diversitree package (FitzJohn et al. 2009, FitzJohn 2012), implemented in R, can incorporate species not sampled in the phylogeny and whether they occur in forest or fynbos. However, as mentioned in the main text, BiSSE performs poorly on phylogenies with less than 300 tips, or a tip-ratio where fewer than 10% of the species is in one of the two states, due to low power (Davis et al. 2013). Our phylogenetic trees of Penaeaceae, Phyliceae and Diosmeae contain 33, 47 and 26 species, and have a tip ratio of 14/19, 2/45 and 1/25 for the forest/fynbos states respectively. We estimated the sampling proportion for species occurring in forest and fynbos for all three clades. For Penaeaceae (including the tropical forest species in the Cryperoniaceae and Alzataceae, as these are the direct sisters of Penaecaeae), the 33-species phylogeny contained 58% of the forest species (14 of 24) and 83% of the fynbos species (19 of 23), the Phyliceae contained 100% of the forest species (2 of 2) and 34% of the fynbos species (45 of 133), and the Diosmeae contained 100% of the forest species (1 of 1) and 9% of the fynbos species (25 of 275).

The full BiSSE model estimates 6 parameters: lambda0, lambda1, mu0, mu1, q01 and q10. State '0' refers to the forest state and state'1' to the fynbos state, and lambda, mu and q to the speciation, extinction and transition rate respectively. We compared the fit of seven alternative models to the full model using likelihood ratio tests. In these alternative models we constrained lambda, mu, q or a combination of these, to be equal between state 0 and state 1. The model with the least number of parameters without having a significantly worse fit than the full model was chosen as the preferred model. This approach may increase the power of the BiSSE algorithm (Davis et al. 2013). Using the preferred model, a MCMC was run on the MCC tree for 10000 generations, using an exponential prior, and a MCMC of 1000 generations for 100 trees was additionally performed to investigate the effect of uncertainty in topology and branch-lengths on the estimated rates. Convergence of the model parameters of the MCMC chains was checked in Tracer 1.5 (Rambaut and Drummond 2007). The diversification rate was calculated as the speciation rate minus the extinction rate, and the posterior probability densities of the diversification rates for state 0 and state 1 were plotted after removing 10% of the generations as the burnin. If the 95% quantiles of the distribution of state 0 and state 1 do not overlap, it is suggested that they can be considered significantly different. However, as indicated by Davis et al. (2013), a BiSSE run with low power can fail in rejecting the null hypothesis of equal rates between states when the alternative hypothesis is true (Type II error).

For Penaeaceae and Phyliceae, a model where the extinction and transition rates were equal for forest and fynbos was preferred, thereby reducing the 6 parameter model to a 4 parameter model. For Diosmeae a model where the extinction rate was equal for forest and fynbos was preferred, reducing the 6 parameter model to a 5 parameter model. We found significantly different diversification rates between forest and fynbos lineages for Penaeaceae, and a tendency for a difference in diversification rates between forest and fynbos lineages for Phyliceae. The probability densities for the diversification rates indicate a higher net diversification rate for fynbos lineages compared to forest lineages in Penaeaceae and Phyliceae. However, the probability density for the diversification rate for the forest state in the Diosmeae could not be estimated accurately, because it is represented by a single lineage (*Calodendrum capense*). The probability densities of the diversification rates for Penaeaceae, Phyliceae and Diosmeae are shown in Figure S1.

Appendix B

Discussion of MEDUSA results.

Penaeaceae:
For Penaeaceae most tips were dropped to genus level, as most genera are well supported (monophyletic)

groups. However, there are two exceptions, the paraphyletic genera *Brachysiphon* and *Stylapterus*. *Brachysiphon* has been fully sampled, and the four tips of *Stylapterus* represent 50% of the species of the genus. Dropping all tips to a multiple-genera tip (approach 3a) is a reasonable approach, but has the main disadvantage that this clade has a very low posterior probability (p.p.= 0.5) and is therefore not represented in all trees (in case of a multiple-tree run). A better solution is to keep all tips of *Brachisiphon* and *Stylapterus* in as they are (these genera may need a taxonomic revision), while all other genera (all with p.p.>0.9) are represented by one tip. The under-sampling of *Stalypterus* is therefore incorrectly ignored, but we think that these 4 species (on a total of 23 within the fynbos clade) will have a minimal effect: firstly, because even with under-sampling of the fynbos clade an increase in rate is detected, and secondly, because these species may cause a more recent (nested) rate shift (by adding more recent splitting events) but the deeper (older) shift (of interest to our hypotheses) is detected in any case.

Phyliceae:
For Phyliceae, all tips were dropped to genus level, as all genera were well supported monophyletic groups.

Diosmeae:
For Diosmeae, most tips can be dropped to genus level, or slightly higher taxonomic clades (e.g. the *Adenandra/ Acmadenia* clade) as most genera or groups are well supported with high posterior probabilities. The only problematic paraphyletic genera are *Agathosma* (150+ species) and *Coleonema* (eight species). Species of these genera appear in two different clades, and dropping them to higher taxonomic levels would collapse the whole fynbos clade (see approaches 4 and 5). We therefore explored the effect of assigning species richness of the genera to one or the other clade, resulting in two (for *Agathosma*) times two (for *Coleonema*) is four different approaches (3a-d). The effect of species assignment of the *Coleonema* species did not have any effect on the location of the diversification rate shifts detected (3a and b versus 3c and d respectively). However, the assignment for *Agathosma* species did have an effect. In the first approach (approach 3a and 3c), the shift is detected at the crown of the fynbos Diosmeae (excluding *Calodendrum capensis*), in the other approach (approach 3b and 3d) the shift is detected within the fynbos Diosmeae (excluding *Calodendrum* and *Phyllosma*) and an additional shift is detected at the stem of *Agathosma*. Although we cannot be entirely sure about the phylogenetic position of *Agathosma*, the first approach (approach 3a) seems most reliable because only one (out of five) *Agathosma* species is "wandering" and ends up in a different clade. All other *Agathosma* form a monophyletic group with *Phyllosma* (but with low support, p.p.=0.67).

Supplemental Table 1. Information and Genbank accession numbers for Phyliceae species sampled.

Taxon name	**ITS**	***TrnL***
Alphitonia excelsa	AF328830.1	AJ390352.1
Ceanothus coeruleus	AF328835.1	
Ceanothus thyrsiflorus	AF328834.1	AJ225798.1
Colubrina asiatica	AF328831.1	AJ390350.1
Colubrina reclinata	AF328832.1	AJ390370.1
Lasiodiscus mildbraedii	AF328833.1	AJ390353.1
Nesiota elliptica	AF328823.1	AJ225803.1
Noltea africana	AF328822.1	KC633945
Phylica abietina		KC633944
Phylica aemula	AF328818.1	AF327618.1
Phylica affinis	KC633914	KC633943
Phylica alba	KC633885	
Phylica ambigua	KC633907	KC633936
Phylica arborea	AF328803.1	AF327603.1

Phylica arborea 108	AF328801.1	
Phylica arborea AI8	AF328802.1	
Phylica axillaris	KC633893	KC633922
Phylica buxifolia	AF328813.1	AF327614.1
Phylica cephalantha	KC633912	KC633941
Phylica cryptandroides	AF328815.1	AF327615.1
Phylica cylindrica	KC633905	KC633934
Phylica disticha	KC633902	KC633931
Phylica ericoides	AF328817.1	AF327617.1
Phylica excelsa	KC633891	KC633920
Phylica fourcadei	KC633908	KC633937
Phylica fruticosa	AF328819.1	AF327619.1
Phylica harveyi	KC633895	KC633924
Phylica humulis	KC633911	KC633940
Phylica imberbis	KC633893	KC633922
Phylica lachnaeoides	KC633901	KC633930
Phylica laevigata	KC633889	KC633918
Phylica laevis	KC633906	KC633935
Phylica lanata	KC633900	KC633929
Phylica lasiocarpa	KC633904	KC633933
Phylica leipoldtia	KC633898	KC633927
Phylica montana	AF328811.1	AF327612.1
Phylica nigrita	KC633910	KC633939
Phylica nigrita2	KC633909	KC633938
Phylica nitida Mauritius	AF328821.1	AJ390356.1
Phylica nitida Reunion	AF328820.1	AF327620.1
Phylica obtusifolia	KC633887	KC633916
Phylica odorata	KC633899	KC633928
Phylica oleaefolia	AF328812.1	AF327613.1
Phylica pani136	AF328808.1	AF327606.1
Phylica pani950	AF328809.1	AF327607.1
Phylica paniculata	AF328807.1	AF327605.1
Phylica paniMag3	KC633884	AF327604.1
Phylica parviflora	KC633903	KC633932
Phylica pinea	KC633888	KC633917
Phylica plumosa	KC633897	KC633926
Phylica polifolia	AF328805.1	KC633915
Phylica polifolia 21	AF328804.1	
Phylica pubescens	AF328814.1	Y16771.1
Phylica purpurea	KC633892	KC633921
Phylica pustulata	KC633896	KC633925
Phylica recurvifolia	KC633913	KC633942
Phylica spicata	AF328816.1	AF327616.1
Phylica thodei	AF328810.1	AF327611.1

Phylica villosa	KC633890	KC633919
Phylica willdenowiana	KC633886	
Pomaderris rugosa	AF328826.1	AJ390363.1
Siegfriedia darwinioides	AF328827.1	AJ390375.1
Spyridium globulosum	AF328828.1	AJ390358.1
Trichocephalus stipularis	AF328825.1	AF327621.1
Trichocephalus stipularis2	AF328824.1	
Trymalium sp1	AF328829.1	AJ390361.1

Supplemental Table 2a: Settings BEAST runs using lognormal tree prior distributions. For settings for the normal tree prior distribution see main text.

	Offset	**Mean**	**Standard deviation**	**95% range**
Penaeaceae	72.8	5	0.5	74.74-82.84
Phyliceae	20.5	5	0.15	24.36-26.82
Diosmeae	45	14	0.4	51.69-69.95

Supplemental Table 2b: Comparing median and HPDs of estimated node ages resulting from two approaches: 1) using **normal** tree prior distributions, and 2) using **lognormal** tree prior distributions.

Penaeaceae

Node (crown)	**Normal**	**Lognormal**
Penaeaceae + Outgroups	77.1 (62.0-94.8)	76.6 (73.9-81.0)
Penaeaceae + *Alzatea*	59.4 (58.8-83.3)	60.5 (48.3-73.7)
Penaeaceae	47.8 (41.5-68.3)	49.2 (37.6-62.2)
Rhynchocalyx + *Olinia*	41.7 (33.9-60.3)	43.3 (31.9-55.0)
Olinia	14.9 (9.4-24.4)	15.1 (8.8-22.7)
Penaeaeae	8.9 (6.2-12.7)	8.8 (6.3-12.0)
C1: *Glischrocolla* + *Endonema*	5.7 (2.2-9.7)	5.7 (2.4-9.7)
Endonema	1.8 (0.2-4.6)	1.8 (0.2-4.6)
Penaeaeae excl. C1	7.8 (5.6-10.9)	7.7 (5.5-10.2)
C2: *Brachysiphon* sp. + *Stylapterus* sp. + *Saltera* + *Sonderothamnus*	7.1 (5.0-10.2)	7.1 (4.9-9.6)
Saltera + *Sonderothamnus*	3.2 (1.4-5.7)	3.2 (1.4-5.4)
Penaeaeae excl. (C1 + C2)	6.3 (4.4-9.5)	6.2 (4.3-8.5)
Penaea sp.	4.6 (2.8-7.0)	4.4 (2.7-6.4)

Phyliceae

Node (crown)	**Normal (HPD)**	**Lognormal (HPD)**
Phyliceae + outgroups	61.4 (42.1-84.7)	52.6 (38.0-69.9)
Phyliceae	22.9(19.4-26.8)	25.5 (24.2-27.1)
Phyliceae excl. *Noltea*	21.4 (17.7-25.0)	23.0 (19.7-25.7)
Nesiota + *Trichocephalus*	16.8 (11.1-22.9)	17.6 (11.8-22.8)
Phylica	15.8 (12.5-19.2)	15.7 (12.5-19.2)
Phylica excl. *P. obtusifolia*	14.7 (11.6-18.0)	14.3 (11.4-17.4)

Diosmeae

Node (crown)	**Normal (HPD)**	**Lognormal (HPD)**
Diosmeae + outgroups	101.1 (55.5-154.0)	103.5 (52.6-150.3)
Excl. *Clausena*	56.8 (42.8-70.9)	57.7 (49.8-69.5)
Diosmeae	47.4 (33.1-62.9)	48.4 (36.0-62.3)
Diosmeae excl. *Calodendrum*	25.2 (16.4-34.9)	25.6 (18.0-35.1)
C1: *Agathosma sp.* + *Phyllosma*	22.0 (13.7-31.9)	22.4 (14.6-32.0)
Diosmeae excl. *Calodendrum* + C1	23.2 (16.41-34.88)	23.6 (18.0-35.1)
Diosmeae excl. (*Calodendrum* + C1 +	21.4 (14.1-30.0)	21.9 (16.7-32.4)

Coleonema sp.)		
C2: *Acmadenia* + *Adenandra*	14.2 (8.2-21.1)	14.4 (8.8-21.4)
Diosmeae excl. (*Calodendrum* + C1 + *Coleonema* + C2)	19.7 (12.9-27.5)	20.2 (14.0-27.8)
C3: *Macrostylis* + *Sheilanthera* + *Empleurum*	17.8 (11.5-25.3)	18.3 (12.3-25.5)
Diosmeae excl. (*Calodendrum* + C1 + *Coleonema* + C2 + C3)	16.8 (10.6-23.7)	17.3 (11.7-24.1)
C4: *Euchaetis*	13.4 (7.9-20.0)	13.9 (8.3-20.1)
Diosma + *Coleonema* sp.	15.3 (9.2-21.8)	15.7 (10.3-22.3)

Supplemental Table 3. Comparing median and HPDs of estimated node ages resulting from two approaches: 1) using a **tertiary calibration** on the Phyliceae crown node, and 2) using two **fossils** to calibrate two nodes in the outgroups. This indicates that the interval of the tertiary calibration is probably a reliable representation of the true age of the Phyliceae.

Node (crown)	**Tertiary calibration (HPD)**	**Fossils (HPD)**
Phyliceae + outgroups	61.4 (42.1-84.7)	36.3 (28.4-68.5)
Phyliceae	**22.9 (19.4-26.8)**	**18.4 (10.3-36.5)**
Phyliceae excl. *Noltea*	21.4 (17.7-25.0)	16.2 (9.4-32.2)
(Noltea+)Nesiota + *Trichocephalus*	16.8 (11.1-22.9)	12.2 (5.6-24.9)
Phylica	15.8 (12.5-19.2)	10.7 (5.8-21.4)
Phylica excl. *P. obtusifolia*	14.7 (11.6-18.0)	9.7 (5.3-19.2)

Supplemental Table 4. MEDUSA approaches, advantages and disadvantages.

Approach	**Description**	**Advantage**	**Disadvantage**
1	Raw: no correction for under-sampling.	Maximum number of splitting events retained, therefore more precise topological placement of the shift is possible.	Under-sampling not considered, therefore unreliable in unbalanced or under-sampled phylogenetic studies.
2	Equal: equal species per genus assignment over tips	Maximum number of splitting events retained, therefore more precise topological placement of the shift is possible.	Under-sampling considered, but assuming random sampling within the genus. Shifts at deeper (old) nodes will therefore be meaningful, but more recent shifts may be arbitrary.
3	Genus-level tips or well supported higher taxonomic groups (including more than one genus).	Under-sampling can be placed with confidence, if the group is well (p.p. $\geq$ 0.9) supported.	Number of splitting events is reduced by dropping tips. However, a trade-off can be made between dropping tips and reliable placement of under-sampled taxa (e.g. if the group has low support, see results Penaeaceae and Diosmeae).
4	Two tips (i.e. Forest-Fynbos)	A specific hypothesis can be tested in this way, e.g. is the the transition from forest to fynbos associated with a shift in the diversification rate?	Number of splitting events is reduced by dropping tips, so information on the exact topological placement of the shift is lacking.
5	Two “ingroup” tips	A specific hypothesis can be tested in this way, e.g. is the the transition from forest to fynbos associated with a shift in the diversification rate? The advantage over the previous approach is that we can distinguish between a stem and crown node shift.	Number of splitting events is reduced by dropping tips, so information on the exact topological placement of the shift is lacking. In addition to the previous approach it has the disadvantage of possibly incorrectly adding information by positioning the crown node of a clade.

Supplemental Table 5. Species richness assignment for the different clades for MEDUSA (example: equal species per genus assignment over tips).

Penaeaceae		**Phyliceae**		**Diosmeae**	
Taxon	n.taxa	taxon	n.taxa	taxon	n.taxa
Brachysiphon acutus	1	*Trichocephalus stipularis*	1	*Acmadenia teretifolia*	11
Brachysiphon fucatus	1	*Nesiota elliptica*	1	*Acmadenia trigona*	11
Brachysiphon microphyllus	1	*Noltea africana*	1	*Adenandra brachyphylla*	11
Brachysiphon mundii	1	*Phylica abietina*	3.02	*Adenandra rotundifolia*	11
Brachysiphon rupestris	1	*Phylica aemula*	3.02	*Adenandra villosa*	11
Endonema lateriflora	1	*Phylica affinis*	3.02	*Agathosma adenandrifolia*	11
Endonema retzioides	1	*Phylica alba*	3.02	*Agathosma bathii*	11
Glischrocolla formosa	1	*Phylica ambigua*	3.02	*Agathosma bifida*	11
Olinia capensis	2.4	*Phylica arborea*	3.02	*Agathosma capensis*	11
Olinia emarginata	2.4	*Phylica axillaris*	3.02	*Agathosma namaquensis*	11
Olinia radiate	2.4	*Phylica buxifolia*	3.02	*Calodendrum capense*	1
Olinia vanguerioides	2.4	*Phylica cephalantha*	3.02	*Coleonema juniperina*	11
Olinia ventosa	2.4	*Phylica cryptandroides*	3.02	*Coleonema pulchrum*	11
Penaea acutifolia	1	*Phylica cylindrica*	3.02	*Diosma hirsuta*	11
Penaea cneorum	1	*Phylica disticha*	3.02	*Diosma oppositifolia*	11
Penaea dahlgrenii	1	*Phylica ericoides*	3.02	*Diosma sabulosa*	11
Penaea mucronata	1	*Phylica excelsa*	3.02	*Diosma subulata*	11
Rhynchocalyx lawsonioides	1	*Phylica fourcadei*	3.02	*Empleurum unicapsulare*	11
Saltera sarcocolla	1	*Phylica fruticosa*	3.02	*Euchaetis glabrata*	11
Sonderothamnus petraeus	1	*Phylica harveyi*	3.02	*Euchaetis glomerata*	11
Sonderothamnus speciosus	1	*Phylica humulis*	3.02	*Macrostylis ramulosa*	11
Stylapterus ericifolius	2	*Phylica imberbis*	3.02	*Macrostylis squarrosa*	11
Stylapterus ericoides	2	*Phylica lachnaeoides*	3.02	*Macrostylis villosa*	11
Stylapterus fruticulosus	2	*Phylica laevigata*	3.02	*Phyllosma capensis*	11
Stylapterus micranthus	2	*Phylica laevis*	3.02	*Sheilanthera pubens*	11
		Phylica lanata	3.02	*Acmadenia obtusata*	11
		Phylica lasiocarpa	3.02		
		Phylica leipoldtia	3.02		
		Phylica montana	3.02		
		Phylica nigrita	3.02		
		Phylica nitida Mauritius	3.02		
		Phylica obtusifolia	3.02		
		Phylica odorata	3.02		
		Phylica oleaefolia	3.02		
		Phylica paniculata	3.02		
		Phylica parviflora	3.02		
		Phylica pinea	3.02		
		Phylica plumosa	3.02		

		Phylica polifolia	3.02		
		Phylica pubescens	3.02		
		Phylica purpurea	3.02		
		Phylica pustulata	3.02		
		Phylica recurvifolia	3.02		
		Phylica spicata	3.02		
		Phylica thodei	3.02		
		Phylica villosa	3.02		
		Phylica willdenowiana	3.02		

Supplemental Table 6. Forest and fynbos plots in the CFR. Locations, GPS coordinates, altitude and transect directions (T), visited in September and October 2011. M=Marloth Nature Reserve, GVB=Grootvadersbosch, GB=Grootbos Nature reserve, MP=Montagu Pass (Outeniqua Nature Reserve), SR=Stormsriver (Garden Route National Park, Tsitsikamma section), Y=Ysternek Nature reserve/Diepwalle Forest (Garden Route National Park, Knysna section), NV=Nature's Valley (Garden Route National Park, Tsitsikamma section), RP=Robinson Pass (Outeniqua Nature Reserve), OB=Oubos (Riviersonderend), TM=Table Mountain (Table Mountain National Park).

Location	Habitat	GPS_S	GPS_E	Latitude (South)	Longitude (East)	Alt	T_1	T_2	T_3	T_4	T_5
M	Forest	33.993	20.451	33°59'34.4394"	20° 27' 4.7154"	310	351	248	342	196	104
M	Fynbos	33.996	20.453	33° 59' 44.952"	20°27'12.5634"	244	273	90	62	15	104
GVB	Fynbos	33.969	20.802	33° 58' 7.80"	20° 48' 7.39"	463	119	138	11	197	122
GVB	Forest	33.985	20.818	33° 59' 4.90"	20° 49' 4.80"	410	192	333	218	148	200
GB	Forest	34.542	19.412	34°32'30.3714"	19°24'44.8914"	207	303	52	294	223	160
GB	Fynbos	34.539	19.411	34° 32' 18.636"	19° 24' 38.592"	201	309	128	216	171	84
MP	Forest	33.887	22.431	33° 53' 13.056"	22°25'51.7794"	691	124	9	308	279	72
MP	Fynbos	33.892	22.427	33°53'29.6874"	22°25'36.8754"	584	306	23	101	330	261
SR	Fynbos	33.962	23.928	33° 57' 42.876"	23°55'39.2874"	261	200	250	257	91	255
SR	Forest	33.964	23.926	33° 57' 50.04"	23° 55' 33.708"	265	275	332	247	205	89
Y	Fynbos	33.926	23.159	33°55'34.1034"	23° 9' 30.9954"	561	11	59	139	90	15
Y	Forest	33.940	23.157	33°56'24.0714"	23° 9' 23.832"	508	59	258	173	239	189
NV	Fynbos	33.969	23.537	33° 58' 6.8154"	23°32'13.5954"	227	158	189	15	130	311
NV	Forest	33.973	23.561	33°58'21.4314"	23°33'40.0674"	47	210	334	253	71	161
RP	Forest	33.890	22.005	33° 53' 25.044"	22° 0' 16.7034"	438	260	120	327	265	-
RP	Fynbos	33.891	22.017	33° 53' 28.14"	22° 1' 0.5154"	785	169	-	-	-	-
OB	Forest	34.079	19.830	34° 4' 45.948"	19°49'46.2354"	346	267	112	262	266	-
OB	Fynbos	34.078	19.836	34° 4' 40.9794"	19° 50' 8.628"	426	190	311	21	262	351
TM	Forest	33.983	18.426	33° 58' 59.736"	18°25'33.1674"	370	91	11	273	334	239
TM	Fynbos	33.989	18.423	33° 59' 20.76"	18°25'24.1314"	292	148	38	192	72	-

Supplemental Table 7. a) BAYESTRAITS settings for correlation tests (function DISCRETE) and b) settings for ancestral node reconstructions (function MULTISTATE, *fossil* node)

a.

Clade		Model	Ratedev	Iterations	Burnin	Prior
Penaeaceae	Habitat-Area	Independent	0.05	5000000	500000	rjhp exp 0.0 30
		Dependent	0.5	5000000	500000	rjhp exp 0.0 30
	Habitat/Area- SLA	Independent	0.1	5000000	500000	rjhp exp 0.0 30
		Dependent	0.5	5000000	500000	rjhp exp 0.0 30
Phyliceae	Habitat-SLA	Independent	2	5000000	500000	rjhp exp 0.0 10
		Dependent	2	5000000	500000	rjhp exp 0.0 10
	Habitat-Area	Independent	2	5000000	500000	rjhp exp 0.0 30
		Dependent	2	5000000	500000	rjhp exp 0.0 10
	Area-SLA	Independent	8	5000000	500000	rjhp exp 0.0 10
		Dependent	8	5000000	500000	rjhp exp 0.0 10
Diosmeae	Habitat-SLA	Independent	10	5000000	500000	rjhp exp 0.0 10
		Dependent	10	5000000	500000	rjhp exp 0.0 10
	Habitat-Area	Independent	0.05	5000000	500000	rjhp exp 0.0 10
		Dependent	10	5000000	500000	rjhp exp 0.0 10
	Area-SLA	Independent	10	5000000	500000	rjhp exp 0.0 10
		Dependent	10	5000000	500000	rjhp exp 0.0 10

b.

Clade	Habitat node	Model	Ratedev	Iterations	Burnin	Prior
Penaeaceae	Penaeaceae all	Fynbos (1)	0.109	10000000	1000000	rjhp exp 0.0 30
		Forest (0)	0.05	10000000	1000000	rjhp exp 0.0 30
	Penaeaeae	Fynbos (1)	0.05	10000000	1000000	rjhp exp 0.0 30
		Forest (0)	0.05	10000000	1000000	rjhp exp 0.0 30
Phyliceae	Phyliceae all	Fynbos (1)	0.09	10000000	1000000	rjhp exp 0.0 30
		Forest (0)	0.4	10000000	1000000	rjhp exp 0.0 30
	Phyliceae expect *Noltea*	Fynbos (1)	0.09	10000000	1000000	rjhp exp 0.0 30
		Forest (0)	0.3	10000000	1000000	rjhp exp 0.0 30
	Phylica	Fynbos (1)	0.5	10000000	1000000	rjhp exp 0.0 30
		Forest (0)	0.3	10000000	1000000	rjhp exp 0.0 30
Diosmeae	Diosmeae all	Fynbos (0)	0.05	10000000	1000000	rjhp exp 0.0 30
		Forest (1)	0.5	10000000	1000000	rjhp exp 0.0 30
	Diosmeae except *Calodendrum*	Fynbos (0)	0.01	10000000	1000000	rjhp exp 0.0 30
		Forest (1)	0.2	10000000	1000000	rjhp exp 0.0 30

Supplemental Table 8.

MEDUSA richness assignment approaches and diversification rate analyses results for Penaeaceae, Phyliceae and Diosmeae. r=net diversification rate (lineages/Myr) and ε=extinction fraction (=μ/λ). Bold analyses indicate that they were run over 100 post-burnin BEAST trees (results in main text and Table 1). a) Penaeaceae, b) Phyliceae, c) Diosmeae.

a.

Penaeaceae: Richness assignment approach	**Root (r)**	**Shift**	**Location**	**< (decrease in r) > (increase in r)**
1. Raw richness	0.0340	0.1961	Penaeaeae crown	>
2. Equal species per genus assignment over tips	r=0.0000139 ε=0.99994	0.2459	Penaeaeae excl. *Endonema* and *Glischrocolla* stem	>
3.a Genus-level tips	0.0424	0.3622	Penaeaeae excl. *Endonema* and *Glischrocolla* stem	>
3.b Genus + species	**0.0395**	**0.1964**	**Penaeaeae crown**	**>**

level tips (4 missing Stylapterus species)				
4. Forest-Fynbos tips	**0.0682**	**0.0501**	**Forest stem (*Olinia+Rhynchocalyx*)**	**<**
5. Two tips ingroup	**0.0395**	**0.2953**	**Penaeaeae crown**	**>**

b.

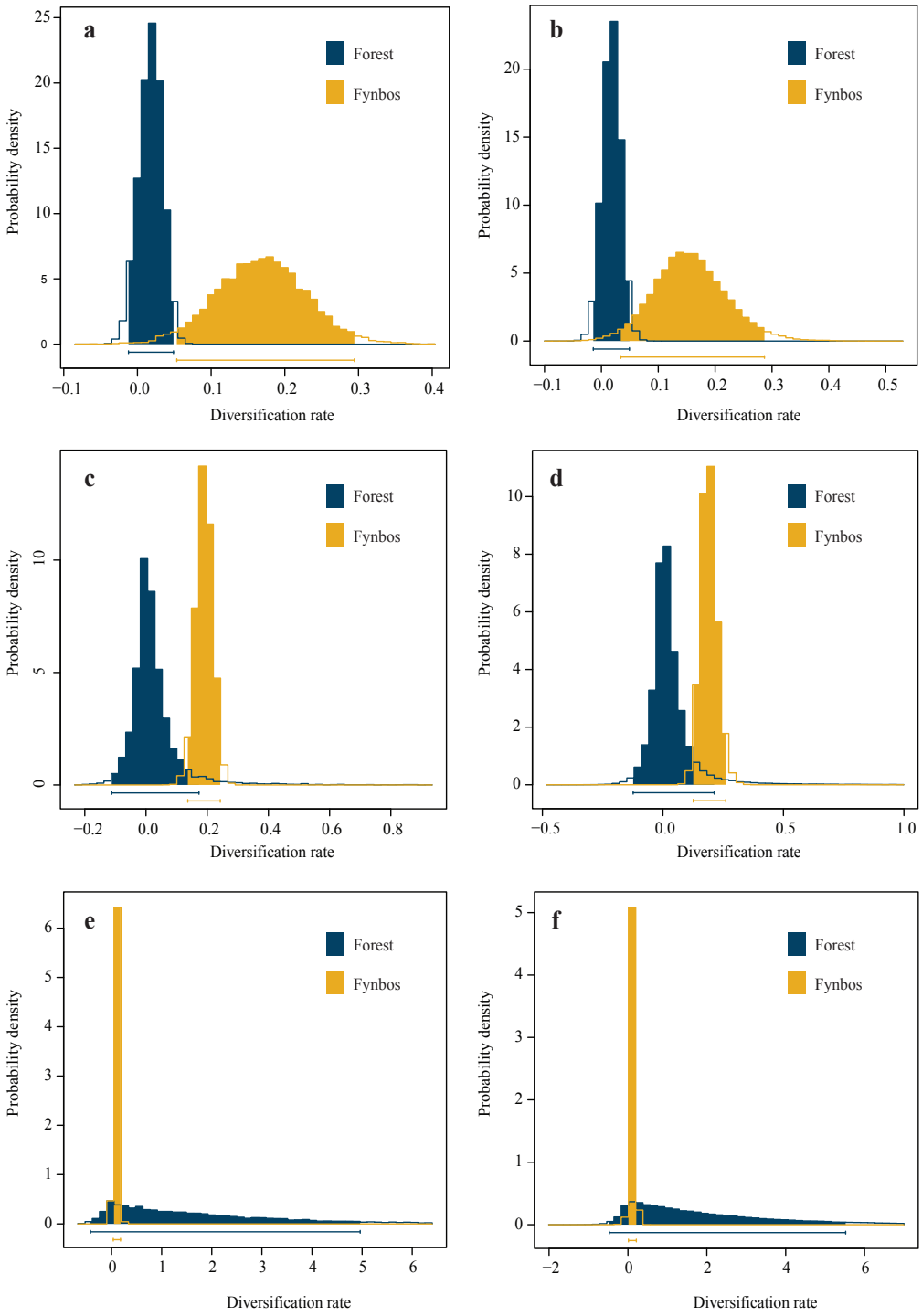
Phyliceae: Richness assignment approach	**Root (r)**	**Shift**	**Location**	**< (decrease in r) > (increase in r)**
1. Raw richness	0.1202	0.0	*Nesiota+Trichocephalus* crown	<
2. Equal species per genus assignment over tips	0.0513	0.2599	*Phylica* excluding *P. obtusifolia* stem	>
3. Genus-level tips	**0.0319**	**0.2283**	***Phylica* stem**	**>**
4. Forest-Fynbos tips	**0.2128**	**0.0479**	**Forest stem (*Noltea/Nesiota/Trichocephalus*)**	**<**
5. Two tips ingroup	**0.0319**	**0.2623**	***Phylica* stem**	**>**

c.

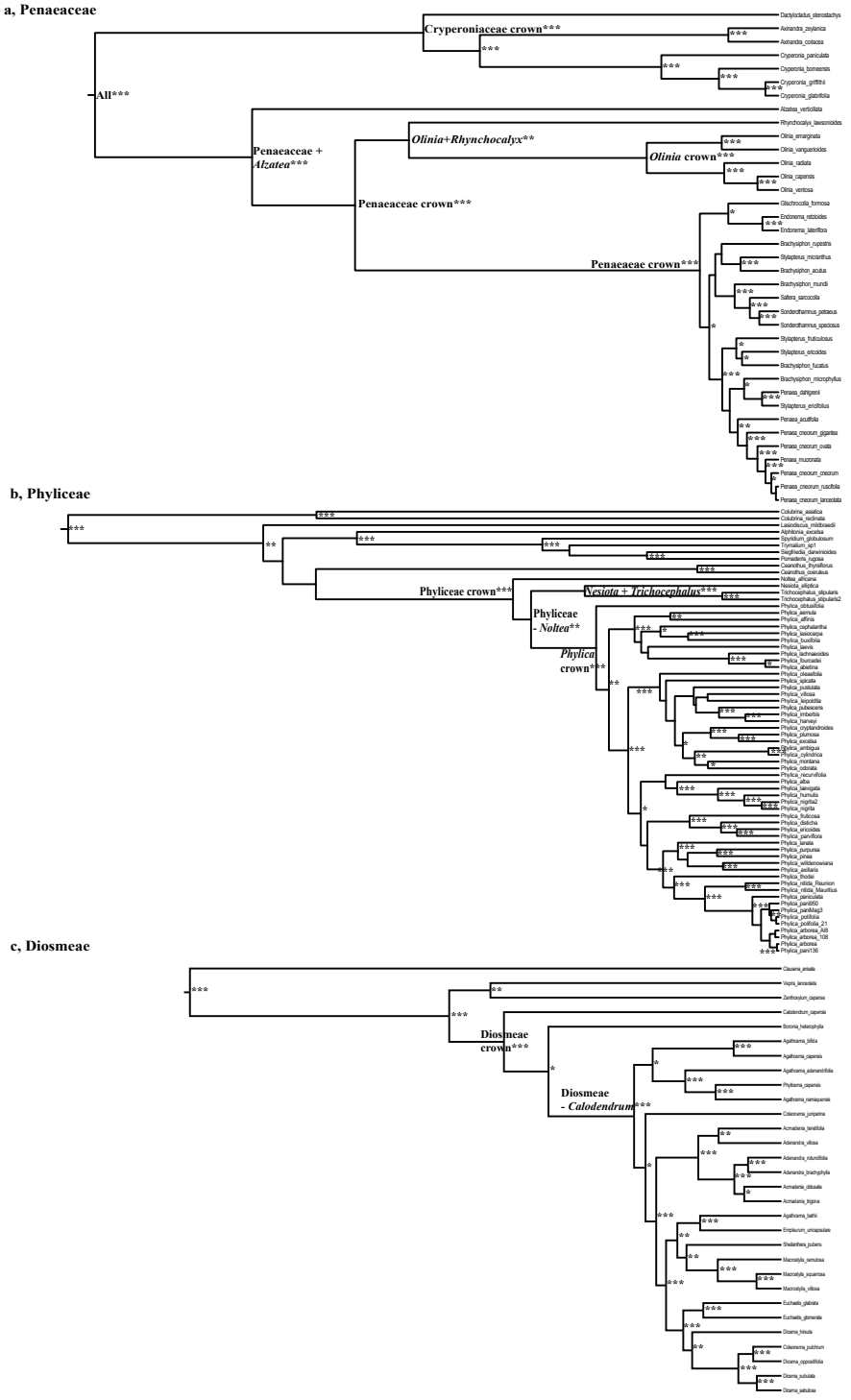
Diosmeae: Richness assignment approach	**Root (r)**	**Shift 1**	**Location**	**< (decrease in r) > (increase in r)**	**Shift 2**	**Location**	**< (decrease in r) > (increase in r)**
1. Raw richness	0.0658	0.0	*Calodendrum* stem	<			
2. Equal species per genus assignment over tips	r=0.1109 **ε**=0.8457	0.0	*Calodendrum* stem	<	0.0	*Sheilanthera* stem	<
3.a Genus-level tips	**0.0144**	**0.1830**	**Diosmeae excl. *Calodendrum* crown**	**>**			
3.b Genus-level tips	0.0188	0.1788	Diosmeae excl. *Calodendrum* and *Phyllosma* stem	>	0.3602	*Agathosma* stem	>
3.c Genus-level tips	0.0144	0.1751	Diosmeae excl. *Calodendrum* crown	>			
3.d Genus-level tips	0.0188	0.1674	Diosmeae excl. *Calodendrum* and *Phyllosma* stem	>	0.3602	*Agathosma* stem	>
4. Forest-Fynbos tips	**0.1184**	**0.0**	**Forest stem (*Calodendrum*)**	**<**			
5. Two tips ingroup	**0.1634**	**-**					

Supplemental Table 9.
MEDUSA AIC/LnLik results for the different approaches on the MCC trees of Penaeaceae, Phyliceae and Diosmeae.

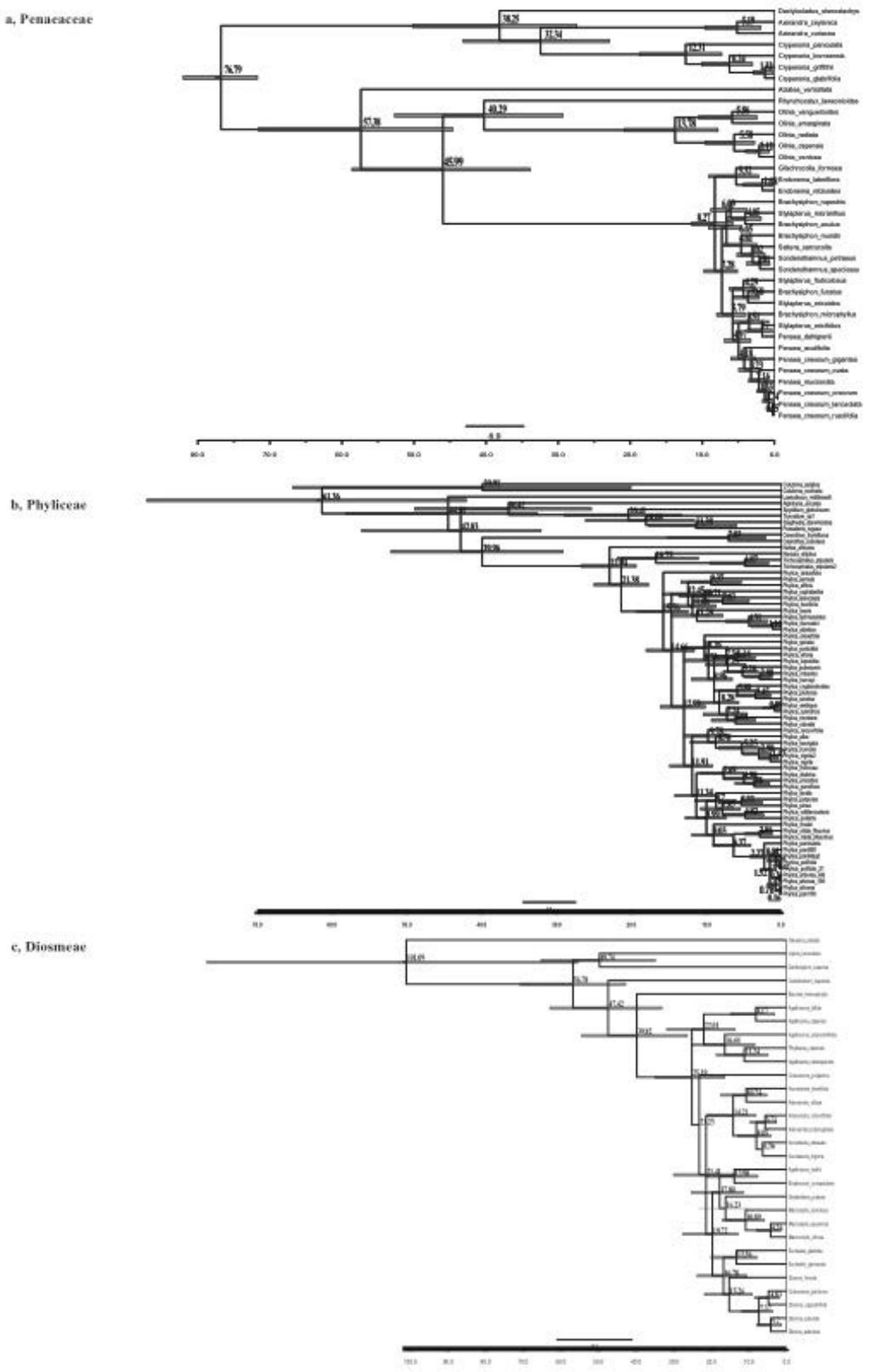
Clade	Approach	Shift	AIC threshold	AICc	lnLik
Penaeaceae	1	No shift	1.647433	226.9748	-111.3906
		one shift		218.4834	-106.045
	2	No shift	1.647433	253.8827	-124.8446
		One shift		248.221	-119.7772
	3a	No shift	0	99.59096	-47.29548
		One shift		93.75689	-42.78754
	3b	No shift	0.369392	176.3383	-86.0263
		One shift		167.8266	-80.62061
	4	No shift	0	21.0897	-7.544852
		One shift		-3.271475	-7.364263
	5	No shift	0	106.7219	-50.86096
		One shift		99.10037	-45.45928
Phyliceae	1	No shift	2.735994	290.3698	-144.1629
		One shift		286.8766	-140.3034
	2	No shift	2.735994	406.8618	-201.3642
		One shift		392.6889	-193.2096
	3	No shift	0	45.45029	-21.32514
		One shift		43.53488	-14.76744
	4	No shift	0	26.81873	-10.40937
		One shift		-2.422906	-7.788547
	5	No shift	0	63.46988	-30.44923
		One shift		54.93691	-22.06846
Diosmeae	1	No shift	0.8278956	186.5727	-92.24554
		One shift		185.1348	-89.31209
	2	No shift	0.8278956	311.5931	-153.6715
		One shift		303.7747	-147.4526
		Two shifts		301.8557	-143.9733
	3a	No shift	0	110.3825	-54.0374
		One shift		99.20902	-45.5136
	3b	No shift	0	131.0534	-63.09815
		One shift		115.1821	-53.66798
		Two shifts		112.6839	-48.61468
	3c	No shift	0	120.6754	-59.20438
		One shift		110.0362	-51.095
	3d	No shift	0	144.0105	-69.63027
		One shift		128.4061	-60.40304
		Two shifts		122.7402	-54.06239
	4	No shift	0	29.69449	-11.84724
		One shift		-4.770099	-6.614951
	5	No shift	0	55.16827	-25.91747



Supplementary Figure 1. Distributions of BiSSE parameter estimates for Penaeaceae (a, b), Phyliceae (c, d) and Diosmeae (e, f). The panels show distributions of estimates of diversification rates (λ−μ) for the MCC trees (a, c, e) and 100 phylogenetic trees (b, d, f) from the Bayesian posterior distribution, from a BiSSE analysis using the model with the least number of parameters without significantly lowering the fit.

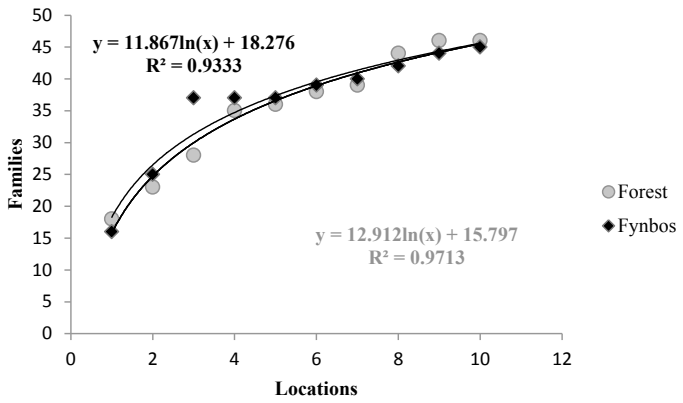


Supplementary Figure 2. Maximum clade credibility trees obtained from 18000 (a, Penaeacaea, c, Diosmeae) and 27000 (b, Phyliceae) post burn-in Bayesian chronograms generated in BEAST. Stars indicate posterior probabilities, *** PP 0.95-1; ** PP 0.75-0.95, * 0.5-0.75.

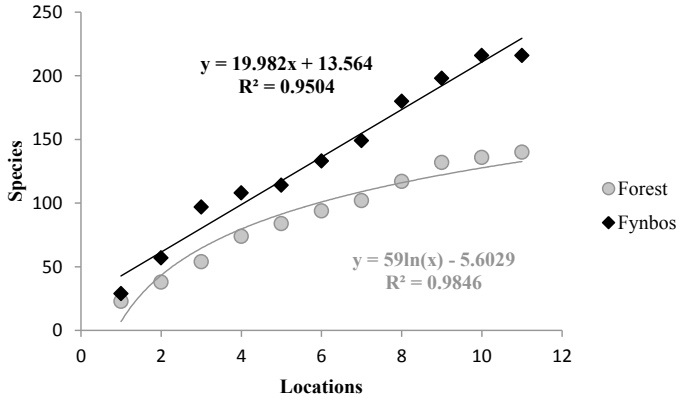


Supplementary Figure 3. Maximum clade credibility trees obtained from 18000 (a, Penaeacaea, c, Diosmeae) and 27000 (b, Phyliceae) post burn-in Bayesian chronograms generated in BEAST. Blue bars at nodes represent 95% Highest Posterior Densities of node ages.

a



b



Supplementary Figure 4. Family and species sampling curves. a) Family sampling curve: additional locations would not add many additional families. b) Species sampling curve: additional forest locations would not add many additional species, but the linear relationship for species sampled relative to plots sampled for fynbos suggests that species remained undetected.

CHAPTER III: DO MEDITERRANEAN-TYPE ECOSYSTEMS HAVE A COMMON HISTORY? – INSIGHTS FROM THE BUCKTHORN FAMILY (RHAMNACEAE)

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Author contributions:

REO and HPL designed the research; RJC assembled the sequence alignment and performed maximum likelihood phylogenetic analyses; YX selected the fossils for calibration; REO collected species distribution data, climate zone data and performed dating and diversification rate analyses; REO wrote the manuscript with major comments HPL and minor comments from YX and JER.

Abstract

Mediterranean-type ecosystems (MTEs) are remarkable in their species-richness and endemism, but the processes which have led to this diversity remain enigmatic. Here, we hypothesize that continent-dependent speciation and extinction rates have led to disparity in diversity between the five MTEs of the world: the Cape, California, Mediterranean Basin, Chile and Western Australia. To test this hypothesis, we built a phylogenenetic tree for 280 Rhamnaceae species, estimated divergence times using eight fossil calibrations and use Bayesian methods and simulations to test for differences in diversification rates. Rhamnaceae lineages in MTEs generally show higher diversification rates than elsewhere, but speciation and extinction dynamics show a pattern of continent-dependence. We detected high speciation and extinction rates in California and significantly lower extinction rates in the Cape and Western Australia. The independent colonization of four out of five MTEs may have occurred conterminously in the Oligocene/Early Miocene, but colonization of the Mediterranean Basin happened later, in the Late Miocene. This suggests that the *in situ* radiations of these clades were initiated before the onset of winter-rainfall in these regions. These results indicate independent evolutionary histories of Rhamnaceae in MTEs, possibly related to the intensity of climate oscillations and the geological history of the regions.

Keywords:

Cape, Californian Floristic Province, diversification rate, extinction, speciation, Western Australia.

Introduction

The five Mediterranean-type ecosystems (MTEs) of the world (the southern African Cape, California-Baja California, the Mediterranean Basin, central Chile and South and Southwest Australia) are exceptional in their species-richness and endemism, containing about 20% of the known vascular plant species, almost 50000, in an area which covers less than 5% of the earth's surface (Cowling et al. 1996). Furthermore, more than 50% of the plant species occurring in MTEs are endemic to MTEs (Greuter 1994, Cowling et al. 1996), which may be due to the geographically and ecologically isolated position of MTEs, enclosed by oceans, deserts and mountain ranges (Cowling et al. 1996, Kottek et al. 2006). The MTEs are geographically separated on different continents (Fig. 1) but are characterized by similar climatic conditions of generally dry, hot summers and cool, wet winters (Aschmann 1973, Castri 1973, Kottek et al. 2006). The processes which resulted in the high *in-situ* diversity are enigmatic (e.g. Linder 2003, Lancaster and Kay 2013). Three processes have been proposed: a higher diversification rate in MTEs than elsewhere, more time to accumulate diversity in these regions than elsewhere, or a high immigration rate into the MTEs (Sauquet et al. 2009, Valente et al. 2010a, Buerki et al. 2012, Buerki et al. 2013, Lancaster and Kay 2013).

Higher net diversification rates (speciation rate – extinction rate) in MTEs compared to other regions could result from increased speciation rates, reduced extinction rates, or a combination of both. Increased net diversification rates were shown for the Proteaceae in the Mediterranean hotspots of Africa (hereafter Cape) and Australia (hereafter Western Australia) compared to tropical environments (Sauquet et al. 2009). However, elevated diversification rates were not detected for the Western Australian *Banksia's* and the Cape *Protea's* (both Proteaceae), which occur in the MTEs as well as in the upland regions of Africa and Eastern Australia respectively, when analysed separately (i.e. without being compared to the background rate of the Proteaceae as a whole) (Valente et al. 2010a, Cardillo and Pratt 2013). The Hyacinthaceae showed higher speciation and higher extinction

rates in the Cape and the Mediterranean Basin than elsewhere (Buerki et al. 2012). In comparison, the diversity in California was shown to result from generally low extinction rates as opposed to elevated speciation rates (Lancaster and Kay 2013). Longer time for speciation in MTEs compared to elsewhere was shown for more species-rich, but also older, Cape lineages compared to younger and species-poorer lineages in the same genus occurring in the Mediterranean Basin (Valente et al. 2011, Valente and Vargas 2013), but 'time-for-speciation' did not affect diversity in the Mediterranean *Dianthus* compared to its African sister clade (Valente et al. 2010b). Finally, higher immigration rates into than out of MTEs may explain diversity in MTEs, but this hypothesis was rejected for most clades and regions (Valente et al. 2011, Cardillo and Pratt 2013, Lancaster and Kay 2013), but see Valente et al. (2010a) and Buerki et al. (2012) for dispersal of *Protea* and Hyacinthaceae respectively into the Cape.

The five MTEs have similarities as well as differences. The comparable climatic conditions in MTEs may have selected for plants with similar functional traits, resulting in analogous vegetation types (Schimper 1903, Specht 1979, Cowling et al. 1996). This 'convergent' functional syndrome is fire-adapted, and includes traits such as a woody, shrubby growth form and often small, evergreen, sclerophyllous leaves (Schimper 1903, Specht and Moll 1983). The dominant vegetation type in all regions is dry shrub- or heathland ('fynbos' in the Cape, 'chaparral' in California, 'kwongan' in Western Australia, 'maquis' in the Mediterranean Basin and 'matorral' in Chile), but extensive areas of sclerophyllous or drought-deciduous forests or woodlands and semi-succulent shrublands can be found as well (Cowling et al. 1996). Although there are many similarities, there are also differences between the regions, such as the geological and geomorphological history, topography, heterogeneity and complexity of the ecosystem, fire frequency or absence (i.e. in Chile), soil nutrient status, biotic elements and the timing of the onset of winter-rainfall (Mediterranean climate) (Thrower and Bradbury 1973, Deacon 1983, Hobbs et al. 1995, Cowling et al. 2005). The Cape and Western Australia have been characterized as relatively stable landscapes compared to the other three MTEs (Cowling et al. 2014). This stability may have resulted from the almost absence of recent orogenic events, subduction or glaciation during the Cenozoic and both these regions have ancient basement complexes, dating back to at least the Paleozoic (Thrower and Bradbury 1973). The exception are two episodes of uplift in southern Africa during the Miocene and Pliocene, lifting the central plateau by ~900 m (Partridge and Maud 1987), these were more pronounced in the Eastern Cape, thereby possibly enhancing summer-aridity in the Western Cape (Tyson and Partridge 2000). The long coastline of the Cape and Western Australia, wedged between two oceans, could have enhanced long-term climatic stability, and even during the Pleistocene the climatic fluctuations in the Cape were minimal compared to the rest of the planet (Meadows and Sugden 1993). Typically, the ancient landsurfaces and the sandy soils have led to leached out, very nutrient-poor soils in these MTEs– therefore called OCBILS ('old, climatically buffered, infertile landscapes', Hopper 2009). This is dramatically different from the other three MTEs (Chile, California, Mediterranean Basin), which are more nutrient rich, and which have been physiographically much more turbulent (Thrower and Bradbury 1973, Hopper 2009). In contrast to the Cape and Western Australia, these areas are marked by relatively young (Late Miocene, Early Pliocene) orogenic events, with mountains rising close to the coast (Thrower and Bradbury 1973). Furthermore, temperature and moisture oscillations associated with Pleistocene glaciations were thought to be more severe in North America, South America and Europe, compared to Australia and South Africa (Markgraf et al. 1995, Farmer and Cook 2013). These differences may therefore have affected the diversification dynamics (i.e. speciation and extinction rates) of the clades evolving in these areas.

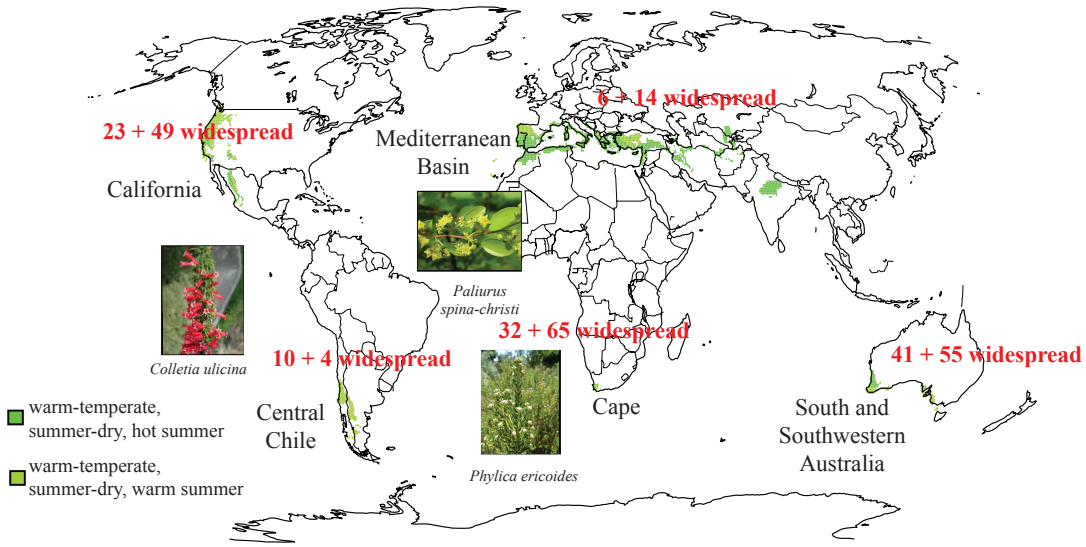

Figure 1
Mediterranean-type ecosystems (MTE) following the Köppen-Geiger climate classification. The number of Rhamnaceae species endemic to the MTE and the number of species widespread (occurring in the MTE as well as outside) are indicated for each region: California, central Chile, the Mediterranean Basin, the Cape and South and Southwestern Australia.

First, we ask why MTEs are generally so diverse, and we hypothesize that overall higher net diversification rates of lineages in MTEs than elsewhere may explain this pattern, as opposed to longer time-for-speciation in MTEs than elsewhere or higher immigration rates into MTEs than vice versa. Second, we test if speciation and extinction dynamics differ between MTEs. We hypothesize that if the winter-rainfall climate is the main factor driving speciation and/or extinction rates (and thus net diversification rates), we would detect similar diversification dynamics in MTEs on different continents, as climate is the consistent factor between the regions. However, if non-climatic factors, which vary between these regions, (additionally) influence speciation and/or extinction rates, we expect to find different, continent-dependent speciation and extinction patterns. To this end, we estimate speciation and extinction rates for each MTE separately, comparing rates of lineages restricted to the respective MTE to rates of lineages occurring elsewhere. Finally, we hypothesize that the timing of colonization of the five MTEs and the accumulation of lineage diversity through time in these areas may explain additional variation in species-richness between the MTEs (i.e. due to time-for-speciation). To this end we reconstruct the first occupation of each MTE to investigate if the colonization of the five MTEs happened in synchrony, and to evaluate how lineage diversity in the five MTEs has varied over time. Such variation could be related to the timing of the onset of winter-rainfall: in the Pliocene (2 – 5 Million years ago (Ma)) in the Mediterranean Basin and California

(Axelrod 1973, Suc 1984, Suc and Popescu 2005), in the mid- to late-Miocene (10 – 15 Ma) in the Cape region (Cowling et al. 2009, Dupont et al. 2011) and similarly in Chile (8 – 15 Ma) (Armesto et al. 2007) and from the early Miocene onwards (20 Ma) in Western Australia (Hopper and Gioia 2004, Martin 2006).

We test these hypotheses in Rhamnaceae Juss. (Rosales), which includes ~1055 species of predominantly warm-temperate shrubs (Medan and Schirarend 2004, also see Supporting Information Table S1). This family is suitable to test these hypotheses for several reasons. First, it occurs in all five MTEs (Fig. 1) - Phyliceae in the Cape, Pomaderreae in Western Australia, *Ceanothus* in California, Rhamneae and Paliureae in the Mediterranean Basin and the Colletieae in Chile - as well as outside these regions. Second, it is phylogenetically well studied (Richardson et al. 2000a, Richardson et al. 2004, Ladiges et al. 2005, Islam and Simmons 2006, Burge et al. 2011) and has a relatively good and well-studied fossil record (Reid and Reid 1915, Basinger and Dilcher 1984, Axelrod 1985, Manchester 2001, Calvillo-Canadell and Cevallos-Ferriz 2007, Burge and Manchester 2008). Finally, Rhamnaceae is often an ecologically important and dominant element in Mediterranean flora's (Axelrod 1973, Linder 2003).

Materials and Methods

Taxon sampling and phylogenetic reconstruction

Sequence data for species of Rhamnaceae and outgroup taxa were collected from GenBank (www.ncbi.nlm.nih.gov) (513 accessions) or generated by ourselves (379 accessions, sequencing procedure and primers in Supporting Information 1, accession numbers are provided in Supporting Information Table S2). We sampled species for six chloroplast markers (*mat*K gene 1593 base pairs (bp), *trn*L-F genes and intergenic spacer 1232 bp, *rbc*L gene 1425 bp, *psb*A gene and *psb*A-*trn*H intergenic spacer 775 bp, *ndh*F gene 2163 bp, *rpl*16 gene and intron 1344 bp) and one nuclear marker (ITS gene 952 bp). All sequences were aligned by Se-Al version 2.0a11 and manually adjusted in Geneious version 5.6 (www.geneious.com). The concatenated alignment contained 59% missing data, but this was not problematic for topological reconstruction or divergence-time estimation (Supporting Information Table S3) (Wiens and Morrill 2011). The final dataset consisted of 307 accessions representing 280 Rhamnaceae species, seven Rhamnaceae subspecies and thirteen outgroup species from related families: Elaeagnaceae, Dirachmaceae and Barbeyaceae. Lineages from all three families were included because the sister family to Rhamnaceae has not been confidently placed yet. All eleven tribes and 50 of the 59 genera were represented by at least one species. At species level, 27% of the currently 1055 recognized species (Supporting Information Table S1) were included.

Phylogenetic reconstruction and dating

Maximum likelihood (ML) analyses were conducted in RAxML version 7.0.4 (Stamatakis 2006) applying the GTR model of sequence evolution with across site rate variation modelled to a Gamma distribution. Gene trees for each marker were generated and manually assessed for incongruence (i.e. conflicting relationships between taxa with >80% bootstrap support), and in absence of incongruence a concatenated alignment was used in all subsequent analyses. Phylogenetically 'unstable' lineages (i.e. the monotypic *Schistocarpea* and *Lasiodiscus*) which may have caused the non-monophylly of the Pomaderreae and the low support for the relationships between the Gouanieae, the Paliureae and the Phyliceae respectively, were removed to investigate the change in relationship and node support. However, as the support for main clades decreased when removing these taxa, and as they are the only representatives of a genus, they were included in all subsequent analyses.

The best fit model for the chloroplast and ITS markers were identified with the Bayesian Information Criterion (BIC) implemented in PartitionFinder v 1.0.1 (Lanfear et al. 2012), as follows:

GTR + G + I for the linked chloroplast markers, and SYM + G + I for the ITS marker. Chloroplast markers were linked to be able to reach convergence in substitution parameters in the dating analysis, which seemed otherwise problematic. The chloroplast and ITS partitions were unlinked for substitution and clock models, but we combined them when estimating the species tree. However, we regressed the median node ages obtained from the combined chloroplast and ITS analysis to the ages when using only chloroplast markers, and results indicate a very strong correlation ($R^2 = 0.96$, Supporting Information Fig. S1). Estimation of Rhamnaceae divergence times was conducted with an uncorrelated lognormal relaxed clock and a yule tree prior available in BEAST v.1.7.5 (Drummond and Rambaut 2007). This was done in two steps. First, we calibrated seven nodes on this tree with fossil-derived mean ages (Supporting Information 2), using normally-distributed priors with a standard deviation of 0.01. The ML tree was used as an input tree. We ran a Markov chain Monte Carlo (MCMC) for 100 million steps, sampling one tree every 2000 steps. Second, we calibrated eight nodes on the tree with fossils (all seven from the previous run, plus one, Supporting Information 2), but using uniform priors, of which the minimum age was indicated by the minimum age of the fossil, and the maximum age by the maximum estimated crown age of Rosales (i.e. 103 Mya, Wang et al. 2009). Uniform priors were chosen to avoid making assumptions about the most probable node age without having additional fossil data (Sauquet et al. 2012). The chloroplast and ITS substitution rate and the yule birth rate mean values as estimated by the previous run were set as initial prior values in this second run. The Maximum Clade Credibility (MCC) tree from the previous run was used as an input tree. Eight independent MCMCs of two times 100 million and six times 30 million steps were conducted, sampling one tree every 2000 steps. Log- and tree-files of the eight chains were combined using LogCombiner v.1.7.5, after removing 20% generations as burn-in, and trees were randomly subsampled to obtain a set of 10250 trees. Tracer v.1.5 (Rambaut and Drummond 2007) was used to evaluate the combined log-file and to obtain the Effective Sample Size (ESS) for each parameter. An ESS of 200 or greater was considered adequate. TreeAnnotator v.1.7.5 was used to obtain the mean node heights, posterior distributions of estimated divergence dates and the 95% highest posterior density (HPD), based on the 10250 subsampled trees, which were mapped on the MCC tree.

Habitat and climate sampling

We obtained distribution data at a 1 × 1 degree resolution for 785 Rhamnaceae species (75% of total species) from the Global Biodiversity Information Facility (GBIF, http://www.gbif.org/) and our own collections and used these distribution data to assign each species to one or more of the 31 Köppen-Geiger climate zones (Fig. 1, Kottek et al. 2006). This classification is based on mean annual temperature, the monthly mean temperature of the warmest and coldest months, the accumulated annual precipitation and the precipitation in the driest month. Species occurring in the climate zones with warm temperate climates with dry summers were coded as present in MTEs, the others coded as absent from MTEs. Occurrence records and species' presence or absence in MTEs were carefully examined for consistency with documented distributions of the species in floras and monographs (Supporting Information Table S1).

Diversification rate analyses

Diversification rate analyses were performed on the Rhamnaceae BEAST MCC tree after pruning outgroups and subspecies. To investigate the effects of topological uncertainty and branch-length variation on the estimated rates all analyses were also conducted on 100 equally spaced subsampled trees from the posterior distribution of the combined BEAST runs. We tested if our phylogenetic sampling of species was a good representation of the total sampling in terms of tribal coverage and presence/absence in MTEs (Supporting Information Fig. S2). To obtain a better proportional coverage of the tribes and their distribution in MTEs in the phylogeny, we randomly pruned lineages of over-

represented clades (i.e. *Phylica* and *Ceanothus*). Analyses were run on the full (i.e. non-pruned, 280 species) as well as the reduced (i.e. pruned, 114 species) phylogeny (hereafter referred to as 'full dataset' and 'reduced dataset'). Finally, to assess the effect of non-fully sampled trees and therefore missing character states on type I and type II errors, we estimated known speciation and extinction rates on simulated trees after pruning lineages with states proportionally to obtain our observed character state proportions.

We tested the effect of MTEs on speciation, extinction and 'dispersal' (immigration) rates with the Geographic State Speciation and Extinction (GeoSSE) algorithm (Goldberg et al. 2011) implemented in the R package 'diversitree' (FitzJohn et al. 2009, FitzJohn 2012). This algorithm can be used to estimate region-dependant rates of speciation, extinction and range evolution based on a fully resolved dated phylogenetic tree and geographical regions assigned to the tips of the tree. Importantly, this algorithm can incorporate species not sampled in the phylogeny and their character states. The sampling proportion for each character state was calculated as the proportion of phylogenetically sampled species for this state divided by the total number of species in the family having this state (Table 1).

Table 1

Number of species for each state in the GeoSSE model: 'both' refers to species occurring in the MTE as well as outside, 'MTE' refers to species restricted in their distribution to the MTE and 'non-MTE' refers to species not occurring in the respective MTE. GeoSSE sampling proportions for the full 280 taxa dataset and the reduced 114 taxa dataset are indicated.

MTE	States	Total species	Species present in phylogeny full dataset	Species present in phylogeny reduced dataset	Sampling proportion full dataset	Sampling proportion reduced dataset
All	both/MTE/non-MTE	188/113/532	117/50/96	80/37/85	0.622/0.442/0.18	0.426/0.327/0.16
California	both/Californian MTEs/ non-Californian MTEs	49/23/870	32/13/231	22/9/179	0.653/0.565/0.266	0.449/0.391/0.206
Chile	both/Chilean MTEs/non-Chilean MTEs	4/10/927	3/2/271	3/2/205	0.75/0.2/0.292	0.75/0.2/0.221
Med. Basin	Both/Med. Basin MTEs/non-Med. Basin MTEs	14/6/857	11/3/255	11/3/189	0.786/0.5/0.298	0.786/0.5/0.221
Cape	both/Cape MTEs/non-Cape MTEs	65/32/851	51/16/203	24/7/178	0.784/0.5/0.239	0.369/0.219/0.209
Australia	both/Australian MTEs/non-Australian MTEs	55/41/862	20/15/240	20/15/174	0.364/0.366/0.278	0.364/0.366/0.202

First, we asked whether speciation, extinction and dispersal vary significantly between lineages occurring in or outside MTEs. We estimated the sampling proportion of species in MTEs, non-MTEs and both regions from 785 Rhamnaceae species, based on their occurrence in the Köppen-Geiger climate zones (Fig. 1). The 280-species phylogeny contained 62% of species that occur in both MTEs and non-MTEs (117 of 188), 44% of only MTE species (50 of 113) and 16% of non-MTE species (96 of 532).

Second, we ask if speciation and extinction rates differ between MTEs. To this end, we ran

five separate GeoSSE models contrasting each MTE against the rest (i.e. 'the rest' includes the other regions with MTEs as well as non-MTE, for sampling proportions see Table 1). The Multiple State Speciation and Extinction (MuSSE) algorithm is able to estimate speciation and extinction rates for a trait with multiple states, and could therefore be used to analyse the MTEs as separate states in a combined analysis. We did not use this approach because, firstly, this model does not consider geographical regions and it is not possible to assign species to more than one state (62% of the Rhamnaceae species occur in MTEs as well as outside). Consequently it may overestimate extinction rates (in the GeoSSE model lineages can only go extinct after being restricted to one of the geographical areas). Secondly, the MuSSE model with six states (five for the MTEs plus one for non-MTE) would estimate 54 parameters (compared to seven GeoSSE parameters): six speciation and six extinction rate parameters, and 42 transition rates. Simultaneously estimating so many parameters with a relatively small dataset may lead to a significant loss of power (Davis et al. 2013). Thirdly, comparing the speciation and extinction rates of lineages occurring in one specific MTE to the remaining Rhamnaceae using the GeoSSE algorithm is unlikely to artifactually produce an apparent pattern of different diversification rates between states; rather, we may fail to reject the null hypothesis of equal rates of diversification, due to the noise caused by the speciation and extinction rate dynamics occurring in the remaining Rhamnaceae lineages.

For all analyses ML parameter estimation and model comparison were conducted followed by Bayesian parameter estimation through MCMC on the MCC trees of the full and reduced datasets. We tested a series of eight models that allowed speciation, extinction and dispersal rates to vary between MTE-lineages and non-MTE-lineages (Supporting Information 3) and selected the best fitted model considering the least number of parameters (assessed by a likelihood-ratio test, Supporting Information 3). This model was then used in the Bayesian MCMC which we ran for 5000 or 10000 steps (depending on the ESS of the parameters), using ML rate estimates as starting points and an exponential prior whose distribution was in relation to the general diversification rate, estimated using the Kendall-Moran estimate for net diversification rate (Kendall 1949, Moran 1951). We evaluated independence of the samples for each parameter in Tracer 1.5 (Rambaut and Drummond 2007). To assess the effect of topological uncertainty on the results, we also ran a MCMC over 100 randomly sampled post-burnin trees of the BEAST analysis for 5000 steps per tree, for the full dataset. If the 5–95 percentiles of the posterior densities of the two rates are non-overlapping, this is strong support for different diversification rates between them (FitzJohn et al. 2009).

Table 2

Median and 95% HPD estimates of rates resulting from the MCMC analyses of the GeoSSE algorithm on the MCC trees of the full dataset, for the analyses combining all MTEs and for each MTE separately. 's' refers to speciation rate, 'x' to extinction rate, 'r' to net diversification rate and 'd' to dispersal rate. Net diversification rates for MTE lineages were calculated as follows: s MTE + ½ × s both – x MTE, and for non-MTE lineages: s non-MTE + ½ × s both – x non-MTE, where 'both' refers to lineages with state 'MTE + non-MTE'.

Analysis	**Selected model**	**s MTE**	**s non-MTE**	**s MTE + non-MTE**	**x MTE**	**x non-MTE**	**r MTE**	**r non-MTE**	**d MTE → non-MTE**	**d non-MTE → MTE**
All	Different speciation rates	0.1478 (0.1217 – 0.1772)	0.0791 (0.0544 – 0.1084)	0.0595 (0.0003 – 0.134)	0.033 (7.86 x 10^{-6} – 0.0732)	0.033 (7.86 x 10^{-6} – 0.0732)	0.1437 (0.0859 – 0.201)	0.0761 (0.0325 – 0.1249)	0.3741 (0.2692 – 0.5117)	0.004 (0.002 – 0.0065)
California	Different speciation and extinction rates	0.4558 (0.3242 – 0.6088)	0.1268 (0.0893 – 0.1676)	0.0911 (0 – 0.3154)	0.4345 (0.2825 – 0.604)	0.0807 (0.0321 – 0.1314)	0.0735 (-0.0543 – 0.2128)	0.0923 (0.0309 – 0.2068)	0.9975 (0.5428 – 1.6105)	0.0041 (0.0017 – 0.0075)
Chile	Same speciation and extinction rates	0.2492 (0.1939 – 0.309)	0.2492 (0.1939 – 0.309)	-	0.1982 (0.1308 – 0.2675)	0.1982 (0.1308 – 0.2675)	0.0515 (0.0345 – 0.0701)	0.0515 (0.0345 – 0.0701)	0.0754 (0.0085 – 0.3038)	0.0003 (8.06 x 10^{-8} – 0.0011)
Med. Basin	Different speciation and extinction rates	0.1182 (0.0483 – 0.2141)	0.1148 (0.0756 – 0.1607)	1.877 (0.9345 – 3.1891)	0.1642 (0.0684 – 0.2895)	0.0944 (0.0265 – 0.1898)	0.8905 (0.4108 – 1.551)	0.9546 (0.4999 – 1.5809)	2.5763 (1.2991 – 4.8652)	0.0036 (0.0014 – 0.0067)
Cape	Different speciation and extinction rates	0.1375 (0.1092 - 0.1675)	0.2131 (0.1657 - 0.2647)	0.0149 (2.12 x 10^{-7} – 0.059)	0.0041 (1.44 x 10^{-7} – 0.0221)	0.1651 (0.1099 – 0.2241)	0.1409 (0.1071 – 0.1786)	0.0571 (0.0375 – 0.0819)	0.4301 (0.252 – 0.6879)	0.0002 (1.09 x 10^{-5} – 0.0004)
Western Australia	Different speciation and extinction rates	0.1018 (0.0754 – 0.1309)	0.2571 (0.1989 – 0.3162)	0.0191 (3.38 x 10^{-6} – 0.0803)	0.005 (4.37 x 10^{-7} – 0.0358)	0.2118 (0.1439 – 0.2764)	0.1052 (0.0621 – 0.1438)	0.0564 (0.0341 – 0.0908)	0.2376 (0.1262 – 0.398)	0.0003 (5.47 x 10^{-7} – 0.0007)

Simulations

Disentangling speciation and extinction parameters using molecular phylogenies is problematic as they co-vary along a ridge of optimal diversification rates, especially for extinction rates (Rabosky 2010). Estimated extinction rates often approach zero, possibly because cladogenetic (speciation) events can be directly inferred from molecular phylogenies, but extinction events cannot (Paradis 2005). Simulations show that the Binary State Speciation and Extinction (BiSSE) algorithm (similar to the GeoSSE algorithm) and the GeoSSE algorithm can accurately estimate rate parameters, including extinction, associated with character states given sufficient sampling of species (Goldberg et al. 2011, Davis et al. 2013). However, phylogenetic trees with too few tips (i.e. less than 300) or with a biased character state ratio (i.e. less than 10% of the species in one state) may give unreliable results (Davis et al. 2013). Rhamnaceae are represented by relatively few lineages in the Mediterranean Basin and Chile, suggesting that our dataset may not have enough statistical power to detect different diversification rates comparing these regions to Rhamnaceae occurring elsewhere, if they exist.

We tested for the possible biased outcome of our dataset by performing two simulation studies, to test for Type I and II errors (FitzJohn et al. 2009, Verdú and Pausas 2013), and results were consistent with the expectations (R scripts are available on request). First, we simulated 100 completely sampled Rhamnaceae trees (1055 species) in which character states evolved following estimated speciation and extinction rates, and then we tested the accuracy by inferring those rates from the trees after pruning 70% of the species, with a proportion to obtain the known character state ratio (i.e. as used in our initial analysis) (FitzJohn et al. 2009). Thereby we test if we fail to reject the false null hypothesis of equal diversification rates between the states (Type II error). We used the estimated median speciation and extinction rates for the lineages occurring in MTEs, non-MTEs and both obtained from the MCMC on the MCC tree, and dispersal rates were chosen to obtain the observed tip (character-) state ratio as known from the literature (i.e. 22% of species occur in MTEs and non-MTEs, 14% is restricted to MTEs and 64% occur outside MTEs). These rates were sA (speciation rate in MTE)=0.148, sB (speciation rate in non-MTE)=0.079, sAB (speciation rate in MTE + non-MTE)=0.06, xA=xB (extinction rate in MTE and non-MTE)=0.033, dA (dispersal rate from MTE to non-MTE)=0.4 and dB (dispersal rate from non-MTE to MTE)=0.004. We then randomly dropped 66% of the lineages with state MTE, 86% of the lineages with state non-MTE, and 51% of the lineages with state MTE + non-MTE, resulting in sampling proportions of 0.336, 0.144 and 0.488 for these states respectively (similar to the known sampling fractions).

In the second experiment, we again simulated 100 completely sampled Rhamnaceae trees (1055 species), but this time we simulated them using the same speciation and extinction rates for both character states, to evaluate if we may incorrectly reject the true null hypothesis of equal diversification rates (Type I error). Dispersal rates were chosen to obtain the tip state ratio as known from the literature (see above), and again we pruned 70% of the species, with a proportion to obtain the character state ratio as we used in the initial analysis. The rates we used were sA=sB=sAB=0.148, xA=xB=0.033, dA=0.5 and dB=0.05, and we randomly dropped 66% of the lineages with state MTE, 85% of the lineages with state non-MTE, and 53% of the lineages with state MTE + non-MTE, resulting in sampling proportions of 0.336, 0.146 and 0.47 for these states respectively (similar to the known sampling fractions). For both simulations studies we then compared the simulated posterior distributions of the diversification rates to the known ones (i.e. our observed posterior distributions).

Time

Finally, we ask if the colonization of the five MTEs happened in synchrony, and how the lineage diversity in the five MTEs varied over time. To this end, we performed a ML ancestral state reconstruction on the Rhamnaceae MCC tree under an equal rate model for the transition between the

discrete states (i.e. non-MTE, California, Chile, Mediterranean Basin, Cape and Western Australia), implemented by the 'ace' function in the 'ape' package in R (Pagel 1994, Paradis et al. 2004). Speciation and extinction may bias ancestral state reconstructions, consequently we compared the results to reconstructions using the speciation, extinction and dispersal rates as estimated by GeoSSE. We used the marginal probabilities of ancestral nodes occupying non-MTE or specific MTEs to estimate the lineage diversity present in that MTE at the time of occurrence of that particular node, using the 'asr' method in the estDiversity function in the 'phytools' package in R (for more details see Mahler et al. 2010, Revell 2012).

Table 3
Timing of colonization of the area currently covered by MTEs by Rhamnaceae lineages based on the ancestral state reconstructions (ASR) for the respective nodes, and the timing of onset of winter-rainfall and the corresponding references for this. The median and 95% HPD of the estimated node age and the posterior probability (p.p.) of the node are indicated.

MTE	Timing of colonization Ma (95% HPD) probability ASR > 0.5	p.p.	Timing of colonization Ma (95% HPD) probability ASR > 0.9	p.p.	Timing of onset winter-rainfall Ma	Reference
Mediterranean Basin	15.0 (8.3 – 22.3)	0.97	8.5 (4.6 – 13.3)	1	2 – 5	Suc (1984), Suc and Popescu (2005)
California	24.4 (16.6 – 34.7)	1	24.4 (16.6 – 34.7)	1	2 – 5	Axelrod (1973)
Chile	28.5 (16.2 – 43.3)	1	28.5 (16.2 – 43.3)	1	8 – 15	Armesto et al. (2007)
Cape	31.1 (22.7 – 41.7)	1	31.1 (22.7 – 41.7)	1	10 – 15	Cowling et al. (2009), Dupont et al. (2011)
Western Australia	43.2 (34.3 – 53.3)	0.99	34.3 (28.1 – 40.9)	1	3 – 20	Hopper and Gioia (2004), Martin (2006)

Results

Phylogenetic relationships

The MCC tree resulting from the dating analyses (Fig. 2a, Supporting Information Fig. S3) is topologically mostly congruent with previously published phylogenetic trees of Rhamnaceae (Richardson et al. 2000a, Richardson et al. 2004, Ladiges et al. 2005, Islam and Simmons 2006, Burge et al. 2011), and includes the most extensive sampling of Rhamnaceae to date. The eleven tribes (Richardson et al. 2000b) were found to be monophyletic with high posterior probabilities (p.p. > 0.9), with the exception of the paraphyletic Pomaderreae and Paliureae (for more details see Supporting Information 4). For discussion on inferred divergence times see 'Discussion'.

Diversification rates in MTEs

For all diversification analyses, the results between the full dataset and the reduced dataset were similar (compare Fig. 3 to Supporting Information Fig. S5), therefore only results using the full dataset are shown here. Results on the MCC trees were similar to results obtained on a set of trees (Fig. 3, Supporting Information Fig. S5), except for Western Australia, where the signal detected on the MCC tree was not retrieved on a set of trees.

We detected higher speciation rates in lineages occurring inside than outside MTEs, resulting in higher net diversification rates for MTE-restricted lineages than non-MTE lineages (Fig. 3, a–c,

Table 2). The best-fit GeoSSE model was a model where the extinction rate of MTE lineages and non-MTE lineages were equal (Supporting Information 3). This model estimated a speciation and diversification rate for lineages occurring in MTEs that is 1.9 times higher than that of non-MTE lineages. The speciation rate for MTE-lineages was estimated to be 2.5 times higher than that of species occurring in MTEs as well as outside of these systems. Relative extinction fractions (extinction rate/speciation rate) are 0.22 in MTEs and 0.42 in non-MTEs. Finally, dispersal rates from MTEs to non-MTEs are significantly higher than dispersal into MTEs. Dispersal into MTEs can therefore not explain the high species diversity in the relatively small areas covered by MTEs (Supporting Information Fig. S4).

Diversification rate dynamics in the five MTEs

Diversification dynamics (speciation and extinction rates) differ between the five MTEs (Fig. 3, d–o, Table 2), but dispersal rates showed a similar pattern for all five MTEs: dispersal from that particular MTE to elsewhere was significantly higher than vice versa, consistent with the generally very low dispersal rate into MTEs (Supporting Information Fig. S5). The best-fit GeoSSE models for each region (Supporting Information 3) included all seven parameters (speciation, extinction and dispersal rates for that particular MTE, for regions elsewhere, and widespread lineages occurring in the MTE as well as elsewhere). The exception was the Chilean model, where speciation and extinction rates for the MTE and non-Chilean areas were similar, and consequently net diversification rates did not differ significantly between Chilean and non-Chilean lineages.

For California, the optimal model specifies a speciation rate for lineages occurring in MTEs that is 3.6 times higher than that of non-Californian lineages and five times higher than that of species occurring in California as well as outside (Fig. 3, d–f). Remarkably, extinction rates are also much higher (5.4 times) in California than outside, resulting in relative extinction fractions of 0.95 in California compared to 0.64 outside. Net diversification rates in California are therefore not significantly different from those outside the region, but these results suggest high turnover of species in California. For the Mediterranean Basin (Fig. 3, g–i), estimated speciation, extinction and consequently net diversification rates were not different from non-Mediterranean Basin areas. However, due to the small number of lineages present in this MTE, and thus relatively low power of the dataset, these results may not reflect true diversification rates in this region. The Cape and Western Australia gave very similar results (Fig. 3 j–o). Speciation rates of lineages restricted to the MTEs were 1.5 times (Cape) and 2.5 times (Western Australia) lower than speciation rates of lineages occurring elsewhere, but were 9.2 times (Cape) and 5.3 times (Western Australia) higher than widespread lineages occurring in the MTEs as well as outside these regions. Extinction rates of lineages restricted to the MTEs were 40 times (Cape) and 42 times (Western Australia) lower than those of lineages occurring elsewhere, resulting in net diversification rates which were 2.5 times (Cape) and 1.9 times (Western Australia) higher within than outside these MTEs. However, the Western Australian signal disappeared when using a set of trees, and this result should therefore be taken with caution (Supporting Information Fig. S5).

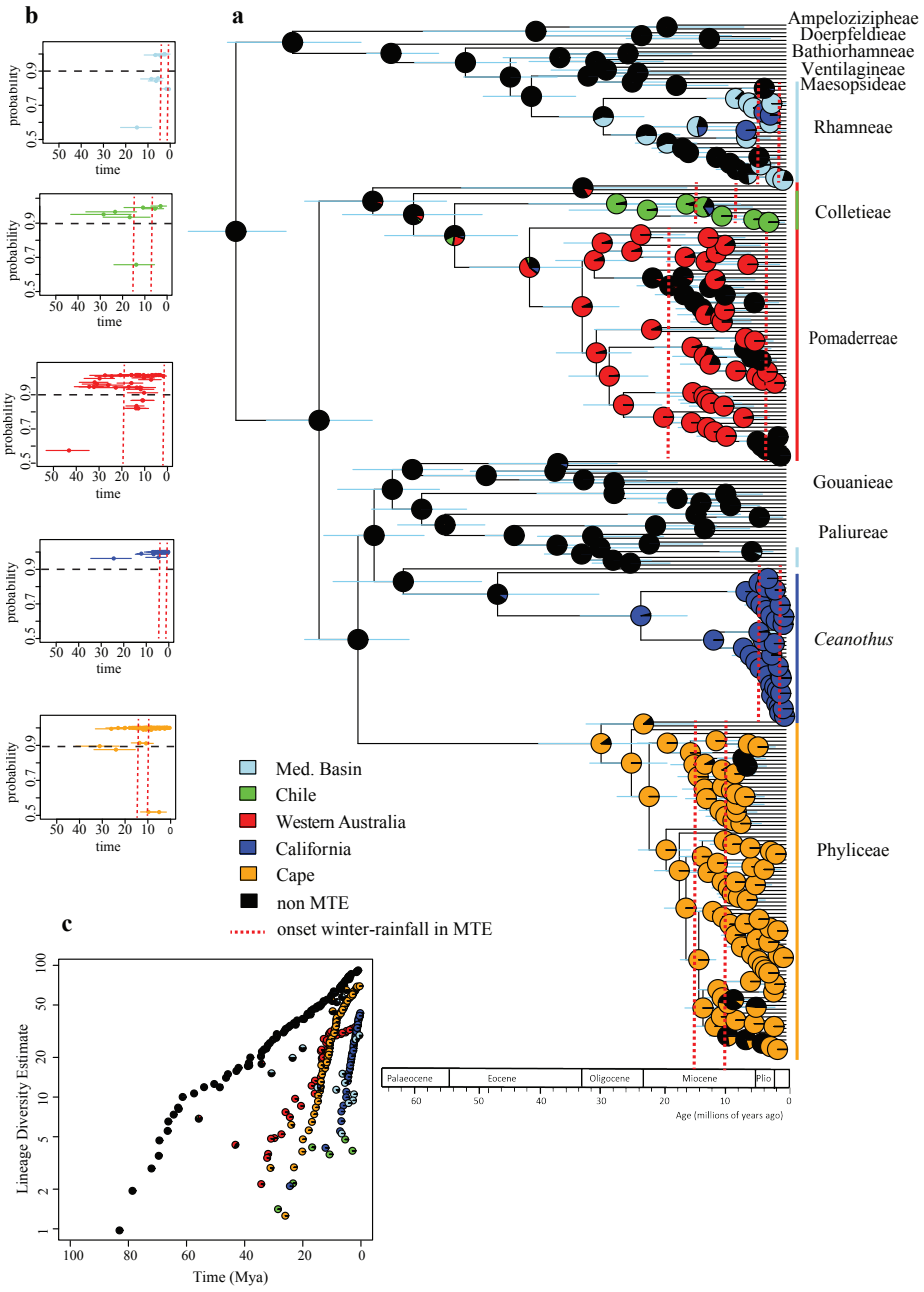

Figure 2

a) Maximum likelihood ancestral area reconstruction under an equal transition rate model (states: California/Chile/Cape/Mediterranean Basin/Western Australia/ non MTE) on the Rhamnaceae MCC tree resulting from the BEAST analysis. Blue bars indicate 95% HPDs of estimated node ages. b) Probability > 0.9 of colonization of each MTE based on the maximum likelihood ancestral area reconstructions in 'a' for each node over time. c) Comparison of historical lineage diversity estimates over time for each node in the Rhamnaceae MCC tree for each MTE. 55% of the extant species occurring in MTEs was sampled, so recent diversity estimates in MTEs (as well as outside MTEs) is underestimated. The colonization of Western Australia, Chile, the Cape and California happened more or less in synchrony (35–20 Mya), but colonization of the Mediterranean Basin happened much later (~8 Mya). Accumulation of lineages was gradual in the Cape and Western Australia, with a slowdown in Western Australia from 10 Mya onwards. Increase in lineage diversity in California happened around 6–8 Mya.

Simulations

Our first simulation study indicated that our dataset had enough power to reject the false null hypothesis of equal diversification rates between the MTE versus non-MTE states and in the second simulation study we were correctly unable to reject the true null hypothesis of equal diversification rates (Supporting Information Fig. S6). This shows that speciation and extinction rates of the character states can be well predicted when all five MTEs are combined into one state, even after pruning 70% of the lineages from the tree.

Time

Ancestral state reconstructions using the GeoSSE parameters agreed with the ML ancestral state reconstructions using an equal rate between states model (Fig. 2a–b, Supporting Information Fig. S7). As the reconstructions based on the equal rate model distinguished between the five MTEs (the GeoSSE parameters were only estimated for MTE versus non-MTE), we will present the results from this method. The dispersal to the areas currently covered by MTEs (Fig. 1) happened more or less in synchrony in Western Australia, Chile, the Cape and California, but could have happened any time between the mid Eocene and the mid Miocene based on 95% HPD values of nodes reconstructed as MTE with a probability > 0.9 (Fig. 2a–b, Table 3). Colonization of the Mediterranean Basin happened later, in the late Miocene. Accumulation of lineages in MTEs in the Cape and Western Australia was steady initially, but a slowdown in Western Australia from 10 Ma onwards was detected. The main increase in lineage diversity in California happened much later, around 6–8 Ma in the late Miocene – Pliocene. No clear pattern of increase in lineage diversity over time was detected in Chile or the Mediterranean Basin (Fig. 2c).

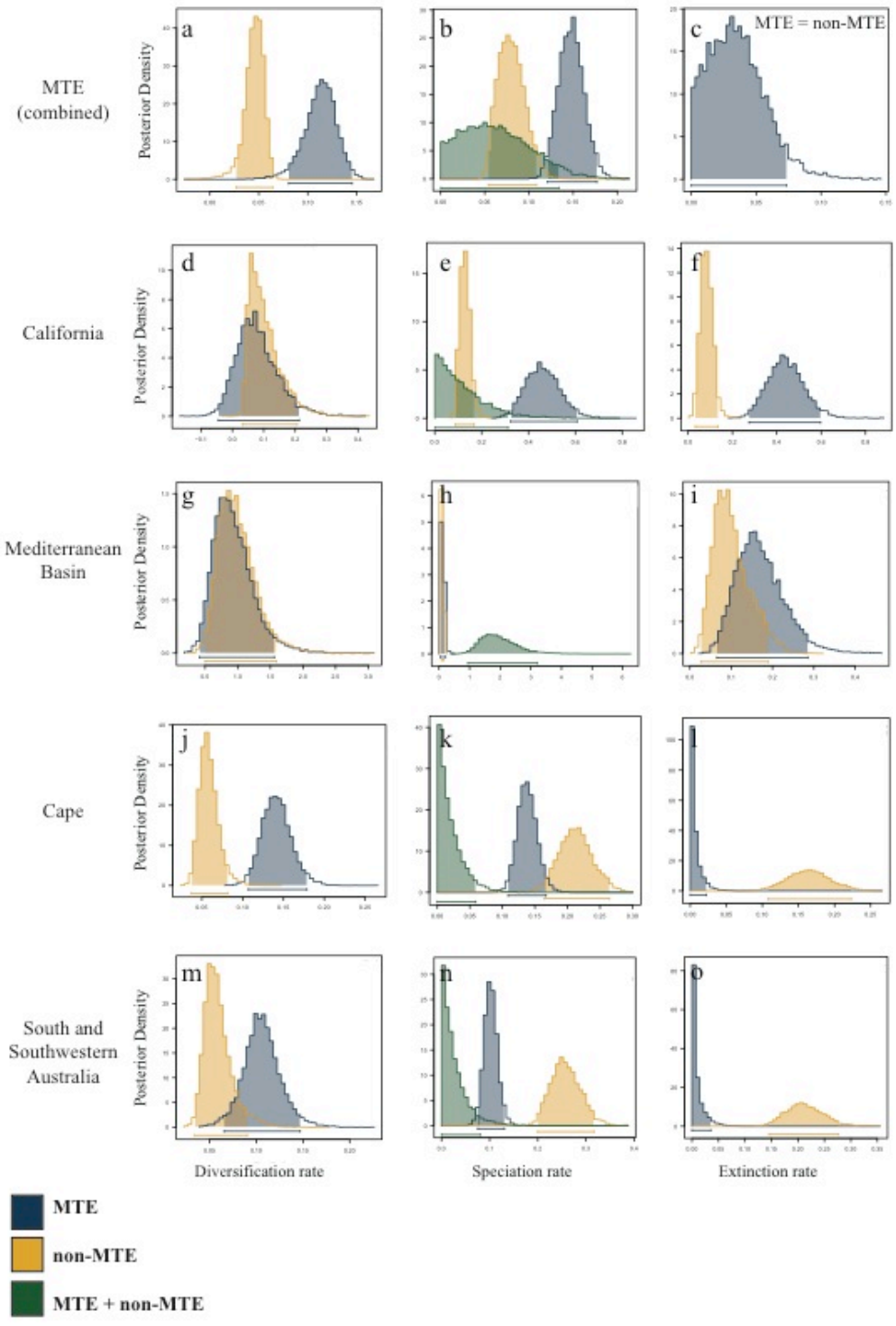

Figure 3

Posterior densities (a–o) resulting from the Bayesian MCMC using the GeoSSE model on the Rhamnaceae MCC tree. Net diversification, speciation and extinction rates for lineages which occur in the Mediterranean-type ecosystems (MTEs) of the world (blue) and outside (yellow), or widespread lineages occurring both in the MTE as well as outside (green) are shown. If only one probability density in a plot is shown it means that the model selected indicated equal rates between MTE/non-MTE for this parameter.

Discussion

We demonstrate here that the extraordinary species-richness of Mediterranean-type ecosystems (MTEs: California, the Cape, Western Australia, central Chile and the Mediterranean Basin) may be, in Rhamnaceae, partly explained by higher diversification rates compared to elsewhere. However, this apparently global signal is mainly driven by high diversification rates in the Cape and possibly Western Australia (Table 2, Fig. 3). Time-for-speciation and dispersal direction unlikely affected Rhamnaceae species-richness in MTEs, as MTE lineages generally derived from non-MTE ancestors (Fig. 2a), and dispersal rates into MTEs were significantly lower than dispersal rates out of MTEs (Supporting Information Fig. S4). However, different evolutionary histories underlie diversification of Rhamnaceae lineages in the five MTEs of the world, as speciation and extinction rate dynamics of MTE-lineages differ between the MTEs (Fig. 3). No significant increase or decrease in speciation or extinction rates could be detected in the Mediterranean Basin and central Chile compared to the outside regions. Lineages occurring in California show higher speciation and extinction rates, suggesting high turnover of species ('ephemeral' species, Rosenblum et al. 2012), compared to lineages occurring outside California, but no difference in net diversification rates was detected between Californian and non-Californian Rhamnaceae. Western Australia and the Cape show a very similar pattern of lower speciation rates, but extremely low extinction rates, resulting in higher net diversification rates, compared to lineages occurring elsewhere, but this pattern is not retrieved for Australia when topological uncertainty and branch-length variation is taken into account. Time to accumulate species in these systems seems not to have affected the species-richness differences between the Cape, Western Australia, Chile and California, as the colonization of these MTEs (or their ancient equivalent) happened approximately at the same time (Table 3, Fig. 2b). However, colonization of the Mediterranean Basin happened later, and time-for-speciation may therefore additionally explain the relatively low Rhamnaceae diversity in this MTE.

Framework for future studies

We presented a methodological framework to assess the impact of limited and biased taxon sampling on estimates of diversification rates in biodiversity hotspots when using molecular phylogenies. Specifically, (1) the use of geo-referenced data to assess taxon distributions, cross-checked with observation from local flora's, allows for the objective assignment of taxa to geographical areas. Furthermore, (2) if all taxa in the clade are sampled for a certain area (also those for which sequence data are unavailable), this allows for sampling proportions to be included in the model (FitzJohn et al. 2009) (Table 1), and for evaluation of sampling biases (Supporting Information Fig. S2). The effects of these on model outcome may be tested (i.e. the full and reduced datasets). And finally, (3) a simulation study could be used to test for type I and II errors on model outcome. We suggest that this framework may be generally useful in diversification rate assessments in species-rich areas where complete species sampling is problematic.

Area as a diversification driver

Area has been suggested to be an important variable impacting diversification rates, and in explaining differences in species-richness between regions (Kisel et al. 2011). The five MTEs differ in area (Fig. 1), and in the ratio between species number and area (Mediterranean Basin with 22,500 species in 2,085,292 km² = 0.01 species/km²; Western Australia with 5500 species in 356,717 km² = 0.015 species/km²; California with 8000 species in 293,804 km² = 0.027 species/km² and the Cape with 9000 species in 78,555 km² = 0.196 species/km²) (Madriñán et al. 2013). Clearly, as the area available for non-MTE lineages is much larger than the area covered by MTEs, this seems not a factor explaining the high species-richness of Rhamnaceae in MTEs compared to elsewhere. In addition,

available area seems not to affect species-richness differences between the five MTEs, where the Cape is exceptional in its high diversity in a relatively small area, compared to the other MTEs. Consequently, we find that area availability does not explain the differences in diversification rates or the standing diversity differences among the MTEs.

Divergence time estimates

Here we estimated crown node ages of 84.1–100.6 Ma (95% HPD, hereafter consistently used for age intervals) for Rhamnaceae. These are substantially older than previous estimates based on molecular data. Bell et al. (2010) used lognormal and exponential prior distributions to estimate crown Rhamnaceae to be 46–73 Ma, and Richardson et al. (2004) estimated it to be even younger: 43.6–49.5 Ma. However, these estimates are in conflict with recently described Rhamnaceae fossils: fossil flowers from Mexico from the late Campanian (~73 Mya) (Calvillo-Canadell and Cevallos-Ferriz 2007) and fossil fruits and leaves from Colombia from the Cretaceous-Maastrichtian (~68 Mya) onwards (Correa et al. 2010), which all show affinities with Rhamneae and Paliureae, tribes within the Rhamnaceae. This suggests that crown Rhamnaceae should at least be 73 Myr old, but probably even older. Furthermore, our Rhamnaceae crown age is in agreement with the Rose Creek fossil flower from the Cretaceous-Cenomanian, ~94 Ma, which shows affinities with Rhamnales as well as with Saxifragales (Basinger and Dilcher 1984, Crepet et al. 2004), and was here used as a minimum age calibration on the root node of Rhamnaceae.

Of central interest in this paper is the dating of the MTE lineages, and here we find agreement with previous studies. There are three recent estimates for the age of the crown Phyliceae, which radiated in the Cape: our study provides the oldest (22.7–41.7 Ma), but shows overlap with previous estimates (19.8–26.7 Ma, Richardson et al. 2004, 10.3–36.5 Ma, Onstein et al. 2014). A similar wide range is available for *Ceanothus* in California: our estimate is the oldest (16.6–34.7 Ma), but overlaps with that of Burge *et al.* 2011 (0.3–26.0 Ma, Burge et al. 2011).

Our use of uniform prior distributions, and setting the maximum age to the presumed age of the Rosales (Wang et al. 2009), may over-estimate the node ages and so underestimate diversification rates of the more recently diversifying MTE clades. However, as we compare diversification rates across the Rhamnaceae, relative rather than absolute estimates of divergence-times are most relevant. The only absolute estimates we use are the colonization times of the MTEs, in comparison to the hypothesized onset of winter-rainfall (Table 3). However, uniform priors, as opposed to exponential or lognormal priors, are known to result in the widest 95% HPDs, and are therefore most conservative (Sauquet et al. 2012). It may be better to consider the whole range of possible posterior outcomes, i.e. the 95% HPD, than using the absolute median estimates.

Diversification under a winter-rainfall climate

The timing of the onset of winter-rainfall was different in the several MTEs, and may have affected the time to accumulate lineages in the systems (time-for-speciation). However, our reconstructions indicate that Rhamnaceae lineages colonized the MTEs before the probable onset of winter-rainfall, even when considering the lower-bound of the 95% HPD of the node age of the node on which the presence in the MTE was reconstructed with a probability of > 0.9 (Fig. 2b, Table 3). There is a consensus among Richardson et al. (2004), Onstein et al.(2014) and Burge et al. (2011) that diversification of Phyliceae and *Ceanothus* started prior to the onset of winter-rainfall in the Cape and California respectively, but possibly during periods of progressive aridification in the regions. The dating of crown groups in the Cape flora prior to the onset of winter-rainfall has been found for several lineages (Verboom et al. 2009, and references therein), with only few radiations postdating the winter-rainfall, most markedly that of Ruschioideae from the semi-arid Namaqualand (Klak et al. 2004) and *Heliophila* (Mummenhoff et al. 2005) also in part from Namaqualand. The ancestral state

reconstructions (Fig. 2a) therefore unlikely reflect the climatic adaptation to winter-rainfall, but rather the colonization of the respective areas.

Nevertheless, it is possible that the generation of the modern diversity was driven by the onset of winter-rainfall, as was demonstrated, for example, in the Mediterranean Basin for *Dianthus* (Valente et al. 2010b) and *Tragopogon* (Bell et al. 2012) among other clades (Fiz-Palacios and Valcárcel 2013), in California for *Linanthus* (Polemoniaceae) (Bell and Patterson 2000), and in Australia and the Cape for Haemodoraceae (Hopper et al. 2009). However the accumulation of Rhamnaceae lineages in the MTEs is constant (linear increase of lineage diversity on a logarithmic scale) and thus seems unaffected by the onset of winter-rainfall (Fig. 2c). Similarly, the hypothesized drying of California in the Pliocene did not seem to affect rates of diversification in most of the sixteen Californian angiosperm clades (Lancaster and Kay 2013). However, few lineages have to date been tested for a diversification rate change in response to the establishment of winter-rainfall (but see Verdú and Pausas 2013).

The slowdown in diversification during the Plio-Pleistocene in Western Australia is puzzling (Fig. 2c), but a similar slowdown was detected in the Australian danthonioids (Linder et al. 2014), which the authors explained by Pleistocene extinctions due to difficulties to move to suitable habitats during periods of climate change, in the topographically subdued Australian landscapes. Alternatively, this pattern may suggest an upper-bound to diversity in Western Australia due to diversity-dependent regulatory mechanisms (Etienne et al. 2011), but this is speculative as clades were under-sampled.

Diversification in the Cape and Western Australia

We show that the high diversification rate of Rhamnaceae in both the Cape and Western Australia is the result of a very low extinction rate (Fig. 3 j-o), which has been suggested for the Cape flora (Cowling and Holmes 1992, Cowling and Lombard 2002, Goldblatt and Manning 2002, Cowling et al. 2004) and for the Western Australian flora (Sniderman et al. 2013, Cowling et al. 2014). The low extinction rate in these old, climatically buffered, infertile landscapes (OCBILS) is proposed to be the result of long-term stable conditions in orogeny and physiography (but see Cowling et al. 2009). The hypothesized long-term climatic stability and gradual aridification in these MTEs, even during the Pleistocene (Meadows and Sugden 1993), could have facilitated diversity (Dynesius and Jansson 2000, Jansson and Dynesius 2002). Cowling et al. (2014) quantified climatic and topographic stability in the five MTEs during the Cenozoic, and showed that the more environmentally stable MTEs (Cape and Australia) have the highest contemporary plant diversity, whereas the least stable (California and Chile) have the lowest diversity. Long-term stability may have led to the evolution of large numbers of highly range-restricted species, with low extinction and low turn-over rates. These differences in orogeny and physiography between the Cape/Western Australia and the other three MTEs (Chile, California, and Mediterranean Basin) are corroborated by our results, indicating continent-dependent speciation and extinction dynamics.

Extant diversity in MTEs

Whether this pattern of speciation and extinction applies to other clades, and may therefore be a general feature of MTEs, remains to be seen. However, there does not seem to be a consensus based on previous studies (see Introduction). The Cape flora is thought to have resulted from both recent (Plio-Pleistocene) and mature radiations, potentially characterized by high speciation rates and low extinction rates respectively, whereas the Western Australian flora is mainly characterized by mature radiations (Linder 2008, Verboom et al. 2009), which seems to apply to the Pomaderreae (Fig. 3 m-o). The high speciation and extinction rates detected for *Ceanothus* in California (Fig. 3 e-f) is different from the general low extinction rates found in Californian clades (Lancaster and Kay 2013), however,

it is comparable to high turn-over rates in Californian lineages non-endemic to serpentine soils, compared to their serpentine-endemic sister clades (Anacker et al. 2011). Furthermore, *Ceanothus* lineages in chaparral vegetation were shown to have higher speciation rates than their Mediterranean forest members (Goldberg et al. 2011), similarly as fynbos *Phylica* to their Cape forest sister *Noltea* (Onstein et al. 2014). This suggests that lineage-specific speciation and extinction rates may be dependent on local factors other than (environmental) stability, variable within MTEs (Goldberg et al. 2011, Onstein et al. 2014).

Conclusions

Here, we show that the extraordinary Rhamnaceae diversity in Mediterranean-type ecosystems (MTEs) has resulted from high net diversification rates rather than from time-for-speciation or from high immigration rates into this biome. However, we show, in contrast to previous ideas (Cox 2001), that the processes leading to this diversity are continent-dependent. The variation in speciation and extinction rates may be the result of intercontinental differences in the orogeny, physiography and climatic fluctuations, and so predated the onset of the common winter-rainfall climate in these regions.

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Supporting Information Chapter III

Supporting Information I

DNA isolation, amplification and primers.

DNA was extracted from species previously collected in the field, from species available in the Botanical Garden in Zurich or DNA extraction were made available to us by the Royal Botanic Gardens in Kew. DNA for *Phylica* was extracted using the 2X CTAB DNA extraction procedure modified from Doyle and Doyle (1987), otherwise the Fast DNA spin kit from MP Biomedicals was used. DNA amplification and sequencing was performed using the primers listed below. Automated sequence output files were assembled into contigs and edited using Sequencer version 4.2 (Gene Codes Corporation), in order to create one consensus sequence for each species by verifying the position of each base with respect to agreement between the two strands.
The species for which data was obtained for at least one marker are listed below, where an 'x' indicates that the marker was successfully sequenced for this species, and collection numbers and location are also indicated.

List of Primers:

Marker	**Primer**	**Direction**	**Sequence**
*trn*L-F	trnF-R	Forward	5`-GAT TTG AAC TGG TGA CAC GAG-3`
	trnL	Reverse	5`-AAA ATC GTG AGG GTT CAA GTC-3`
ITS	ITS-Leu	Forward	5`-GTC CAC TGA ACC TTA TCA TTT CG-3`
	ITS-4	Reverse	5`-TCC TTC CGC TTA TTG ATA TGC-3`
*psb*A and psbA-trnH	psbA-F	Forward	5`-GTT ATG CAT GAA CGT AAT GCT C-3`
	trnH-R	Reverse	5`-CGC GCA TGG TGG ATT CAC AAA TC-3`
*ndh*F	ndhF-1B	Forward	5`-CCT TYA TTC CRG TTC CAG TTC C-3`
	ndhF-8B	Reverse	5`-ATA GAT TCG ACA CAT ATA AAA TGC AGT T-3`
*rpl*16	Rpl16-F71	Forward	5'-GCTATGCTTAGTGTGTGACTCGTTG-3'
	Rpl16- R1516	Reverse	5'-CCCTTCATTCTTCCTCTATGTTG-3'

Markers, collection numbers (CollNr) and location for species sampled:

Tribe	Species	CollNr	ITS	*trnL*	*ndh F*	*rpl* 16	*psb A*	Location
Colletiaeae	*Colletia hystrix*	CW2599				x		Kew
	Colletia spinosa Lam.	CW12	x	x	x	x		Botanical Garden Zurich
	Colletia paradoxa	CW1052				x		Kew
	Colletia ulicina	CW608				x		Kew
	Discaria chacaye	CW914				x		Kew
	Pleuranthodes hillebrandii	CW2379		x				Kew
Paliureae	*Hovenia acerba*	CW08		x	x	x		Botanical Garden Zurich
	Hovenia dulcis	CW16			x	x		Botanical Garden Zurich
	Paliurus spina-christi	CW05		x	x	x		Botanical Garden Zurich
	Ziziphus jujuba Mill.	CW06						Botanical Garden Zurich
	Nesiota elliptica	CW500		x				Kew
	Phylica acmaephylla	Haemmerli S 173	x	x	x	x	x	Western Cape (WC)
	Phylica aemula	Haemmerli S 67	x	x		x	x	WC
Phyliceae	*Phylica alba*	Haemmerli S 142		x	x	x	x	WC
	Phylica altigena	Haemmerli S 201	x	x	x	x		WC
	Phylica ambigua	Haemmerli S 119			x	x		WC
	Phylica apiculata	Haemmerli S 167		x	x	x		WC
	Phylica atrata	Haemmerli S 41	x	x	x	x	x	WC
	Phylica axillaris	Haemmerli S 147		x	x	x		WC
	Phylica buxifolia	Haemmerli S 98			x	x	x	WC
	Phylica callosa	Haemmerli S 102		x	x		x	WC
	Phylica cephalantha	Haemmerli S 155		x	x			WC
	Phylica confusa	Haemmerli S 12	x	x	x			Eastern Cape (EC)
	Phylica constricta	Haemmerli S 52	x	x	x	x	x	WC
	Phylica cryptandroides	Linder H P 7900	x	x	x	x		WC
	Phylica curvifolia	Haemmerli S 25	x		x	x	x	WC
	Phylica cuspidata	Linder H P 7899	x	x	x	x		WC
	Phylica cuspidata minor	Haemmerli S -						WC
	Phylica cylindrica glabrata	Haemmerli S 120			x			WC
	Phylica debilis debilis	Haemmerli S 30	x	x		x	x	WC
	Phylica debilis fourcadei	Haemmerli S 13	x	x		x		EC
	Phylica dioica	Haemmerli S 47	x	x	x	x		WC
	Phylica diosmoides	Haemmerli S 79	x	x	x	x	x	WC
	Phylica disticha	Haemmerli S 194			x	x		WC
	Phylica dodii	Haemmerli S 92		x	x			WC
	Phylica elsieae	Haemmerli S 51	x		x	x	x	WC
	Phylica emirnensis	Bellstedt D 1305				x	x	-
	Phylica empetroides	Haemmerli S 80	x	x	x	x	x	WC
	Phylica ericoides	Haemmerli S 43		x	x	x		WC

	Phylica excelsa	Haemmerli S 57		x		x	x	WC
	Phylica floribunda	Haemmerli S 182			x	x		WC
	Phylica fourcadei	Haemmerli S 16				x		WC
	Phylica fruticosa	Haemmerli S 200		x	x	x	x	WC
	Phylica gnidioides	Haemmerli S 9	x	x		x	x	EC
	Phylica gracilis	Haemmerli S 105	x	x	x	x		WC
	Phylica harveyi	Haemmerli S 197			x	x		WC
	Phylica humilis	Haemmerli S 212			x	x	x	WC
	Phylica imberbis	Haemmerli S 36			x	x	x	WC
	Phylica incurvata	Haemmerli S 185	x	x	x	x	x	WC
	Phylica intrusa	Haemmerli S 62	x	x	x	x	x	WC
	Phylica karroica	Haemmerli S 18	x	x		x		WC
	Phylica keetii	Haemmerli S 14	x	x			x	EC
	Phylica laevigata	Haemmerli S 148			x		x	WC
	Phylica laevis	Haemmerli S 83		x	x		x	WC
	Phylica lanata	Haemmerli S 137			x	x	x	WC
	Phylica lasiantha	Haemmerli S 154	x	x	x	x	x	WC
	Phylica lasiocarpa	Haemmerli S 40			x	x	x	WC
	Phylica leipoldtii	Haemmerli S 203			x	x		WC
	Phylica levynsiae	Haemmerli S 206	x	x		x	x	WC
	Phylica litoralis	Haemmerli S 10	x	x		x	x	EC
	Phylica lucens	Haemmerli S 88	x	x	x	x	x	WC
	Phylica meyerii	Haemmerli S 20	x	x		x	x	WC
	Phylica minutiflora	Haemmerli S 94			x	x		WC
	Phylica nigrita	Haemmerli S 38		x	x	x	x	WC
	Phylica nitida	Ah-Peng C 688		x	x	x		-
	Phylica obtusifolia	Haemmerli S 61			x	x		WC
	Phylica odorata	Haemmerli S 66			x	x	x	WC
	Phylica oleaefolia	Haemmerli S 112			x	x		WC
	Phylica paniculata	Haemmerli S 134				x		WC
	Phylica parviflora	Haemmerli S 44			x	x	x	WC
	Phylica parvula	Haemmerli S 187	x	x	x	x	x	WC
	Phylica pauciflora	Haemmerli S 205	x	x	x	x		WC
	Phylica pinea	Haemmerli S 26			x	x	x	WC
	Phylica plumosa	Haemmerli S 110			x	x		WC
	Phylica propinqua	Haemmerli S 152	x		x	x		WC
	Phylica pulchella	Haemmerli S 204	x	x	x	x		WC
	Phylica purpurea pearsonii	Haemmerli S 33	x	x	x	x	x	WC
	Phylica purpurea purpurea	Haemmerli S 153	x	x	x	x		WC
	Phylica pustulata	Haemmerli S 113			x	x		WC
	Phylica rigida	Haemmerli S 65	x	x	x	x		WC
	Phylica rogersii	Haemmerli S 55	x	x	x	x	x	WC
	Phylica rubra	Haemmerli S 180	x	x	x	x		WC
	Phylica selaginoides	Haemmerli S 164	x	x	x	x		WC
	Phylica sericea	Haemmerli S 138	x	x	x	x		WC

	Phylica stenantha	Haemmerli S 56	x	x	x	x	x	WC
	Phylica strigosa	Haemmerli S 49	x	x	x	x	x	WC
	Phylica thodei	Gehrke B 571			x	x		WC
	Phylica thunbergiana	Haemmerli S 103		x	x	x	x	WC
	Phylica trachyphylla	Pirie M 529	x	x	x	x		WC
	Phylica villosa	Haemmerli S 118			x	x		WC
	Phylica virgata	Haemmerli S 53	x		x	x	x	WC
	Phylica vulgaris major	Haemmerli S 59	x	x	x	x	x	WC
	Phylica vulgaris vulgaris	Haemmerli S 131	x	x	x	x		WC
	Phylica wittebergensis	Haemmerli S 31	x	x		x	x	WC
	Trichocephalus stipularis	Haemmerli S 73				x		WC
	Berchemia racemosa	CW07		x	x	x	x	Botanical Garden Zurich
	Frangula rupestris	CW14	x	x	x		x	Botanical Garden Zurich
Rhamneae	*Rhamnus alaternus*	CW04			x	x	x	Botanical Garden Zurich
	Rhamnus fallax	CW01	x	x	x	x		Botanical Garden Zurich
	Rhamnus lycioides	CW11					x	Botanical Garden Zurich
	Rhamnus pumila	CW15			x	x		Botanical Garden Zurich
	Rhamnus purshianus	CW10	x	x	x		x	Botanical Garden Zurich
	Rhamnus saxatilis	CW09			x		x	Botanical Garden Zurich
	Rhamnus sphaerospermus Sw. var. pubescens.	CW03		x	x		x	Botanical Garden Zurich
	Rhamnus taquetii	CW02	x	x	x	x	x	Botanical Garden Zurich

Supporting Information 2

Fossil selection and BEAST settings.

Rhamnaceae fossils used for calibration in BEAST:

	Node	**Fossil name**	**Locality**	**Age (Mya)**	**Reference**
1	Rhamnaceae stem	Rose Creek flower	Dakota Formation, Nebraska	94 – 96	(Basinger and Dilcher 1984)
2	Ziziphea (Rhamneae/Paliureae) crown	*Coahuilanthus belindae*	El Almácigo, Cerro del Pueblo Formation, General Cepeda County, Coahuila, Mexico	70.6 – 83.5	(Calvillo-Canadell and Cevallos-Ferriz 2007)
3	*Paliurus* stem	*Paliurus clarnensis*	Red Gap, Jefferson County, Oregon	ca. 44	(Burge and Manchester 2008)
4	*Ventilago* stem	*Ventilago engoto* sp. nov.	Los Ahuehuetes, Coatzingo Formation, Puebla, Mexico	23 – 33.9	(Calvillo-Canadell and Cevallos-Ferriz 2007)
5	*Ceanothus* crown	*Ceanothus precuneatus*	Middlegate, USA	16 – 20	(Axelrod 1985)
6	*Ceanothus* subgenus *Ceanothus*	*Ceanothus leitchii*	Purple Mountain Flora, USA	11.6 – 14	(Axelrod 1995)
7	*Colubrina* crown	*Colubrina spireaefolia*	Florrisant, USA	34 – 37.2	(Manchester 2001)
8	*Karwinskia* stem	*Karwinskia paucicostata*	Reuver, the Netherlands	2.6 – 3.6	(Reid and Reid 1915)

1. The Rose Creek flower shares features with different orders of extant plant lineages, but Richardson et al. (2000a) conclude that it is "clearly a member of Rhamnaceae with obhaplostemonous flowers and ''rhamnaceous'' pollen." The fossil was found at the Dakota formation, Nebraska, and was dated to 94 – 96 Mya. Because it also shares morphological features with for example the Saxifragales and other Rosales families (Basinger and Dilcher 1984) we placed it at the stem of the Rhamnaceae.

2. The floral morphology of *Coahuilanthus belindae* is consistent with Rhamneae (e.g., *Rhamnu*s and *Sageretia*) and Zizypheae (e.g., *Berchemia*) based on floral cup structures and parts of the perianth (e.g. five acute, triangular, slightly keeled sepals) (Calvillo-Canadell and Cevallos-Ferriz 2007). We therefore placed it on the common ancestor of the Rhamneae and Paliureae, which is the crown of the Rhamnaceae.

3. This is the oldest known fossil (fruit) for *Paliurus*. It differs from other known fossil forms of *Paliurus*, for example, it has RD allometry. It may therefore represent an ancestral or early diverging lineage of the genus, and was placed on the stem of *Paliurus* (Burge and Manchester 2008).

4. The fossil fruit shares characteristics with extant Ventilaginea: a narrowly elliptic form, rounded base, elliptic wing with reticulate venation, a mid-vein that passes through the wing fruit, a nut-like structure, and the symmetric distal wing attached to a basal seed chamber. Due to the single Ventilaginea taxon in our tree, it was placed on the stem of this lineage (Calvillo-Canadell and Cevallos-Ferriz 2007).

5. This *Ceanothus* fossil species was classified based on a single fossil leave. Though it has been assigned to an extant species, we decided that there are not enough characters to distinguish it from all other extant species. It was therefore placed at the crown of *Ceanothus*.

6. This *Ceanothus* fossil species has been found from several Neogene fossil floras in North America. It was believed to belong to subgenus Ceanothus and was assigned to an extant species. However, no detailed comparisons have been made and it may also be morphologically similar to other species in the subgenus. We therefore placed it at the crown of *Ceanothus* subgenus *Ceanothus*.

7. This *Colubrina* fossil species was discovered from the late Eocene Florrisant Beds based on several well preserved fossils leaves. The fossil species differs from other rhamnaceous genera such as *Ceanothus, Paliurus* and *Zizyphus* by lacking numerous secondary branches from the three primaries and the coarse marginal dentations which is only existed in the genus *Colubrina*. The fossil species shows similarities with two extant species *C. arborea* and *C. glomerata* but differ in having more numerous and more acute marginal dentations (MacGinitie 1953). Therefore, we place it at the crown of *Colubrina*.

BEAST settings

BEAST step 1, fossil calibrations

Calibration	**Prior**	**Stem/Crown**	**Monophyletic?**
Rhamnaceae stem	Normal 94	Stem	Yes
Ziziphea (Rhamneae/Paliureae) crown	Normal 70.6	Crown	No
Paliurus stem	Normal 44	Stem	Yes
Ventilago stem	Normal 23	Stem	Yes
Ceanothus crown	Normal 16	Crown	Yes
Ceanothus subgenus Ceanothus	Normal 11.6	Crown	Yes
Colubrina crown	Normal 28.4	Crown	Yes
Karwinskia stem	Normal 2.6	Stem	Yes

BEAST step 2, fossil calibrations

Calibration	**Prior**	**Stem/Crown**	**Monophyletic?**
Rhamnaceae stem	Normal (94, 20) 0-103	Stem	Yes
Ziziphea (Rhamneae/Paliureae) crown	Uniform 70.6-103	Crown	No
Paliurus stem	Uniform 44-103	Stem	Yes
Ventilago stem	Uniform 23-103	Stem	Yes
Ceanothus crown	Uniform 16-103	Crown	Yes
Ceanothus subgenus Ceanothus	Uniform 11.6-103	Crown	Yes
Colubrina crown	Uniform 28.4-103	Crown	Yes
Karwinskia stem	Uniform 2.6-103	Stem	Yes

BEAST step 2, prior settings for chloroplast and ITS mean substitution rates, and the yule birth rate

Parameter	**Prior**
Chloroplast subst. rate (mean)	Lognormal initial value: 4.573E-4, mean= 4.573E-4, stdev=0.5 (Mean in Real Space)
ITS subst. rate (mean)	Lognormal initial value: 0.0022383, mean= 0.0022383, stdev=0.5 (Mean in Real Space)
Yule birth rate	Lognormal initial value: 0.074737, mean= 0.074737, stdev=0.5 (Mean in Real Space)

Supporting Information 3

Model testing GeoSSE.

'd' refers to 'dispersal rate', 's' to 'speciation rate' and 'x' to 'extinction rate'.
'Df'=degrees of freedom, lnLik=log-likelihood, AIC=Akaike Information Criterion, ChiSq=Chi square. In **bold** the selected model.

MTE (all)

Full dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
Full	7	-1248.9	2511.8		
equal.d	6	-1312.3	2636.5	126.761	< 2.2e-16 ***
equal.s	5	-1251.2	2512.4	4.641	0.09821 .
equal.x	**6**	**-1250.1**	**2512.1**	**2.362**	**0.12434**
equal.sxd	3	-1361.7	2729.4	225.659	< 2.2e-16 ***
equal.sx	4	-1266.0	2539.9	34.125	1.864e-07 ***
equal.sd	4	-1322.2	2652.5	146.701	< 2.2e-16 ***
equal.xd	5	-1328.6	2667.3	159.500	< 2.2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Reduced dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	7	-999.67	2013.3		
equal.d	6	-1044.57	2101.2	89.803	< 2.2e-16 ***
equal.s	**5**	**-999.78**	**2009.6**	**0.213**	**0.89912**
equal.x	**6**	**-1001.47**	**2014.9**	**3.595**	**0.05795 .**
equal.sxd	3	-1079.17	2164.3	158.992	< 2.2e-16 ***
equal.sx	4	-1012.93	2033.9	26.524	7.409e-06 ***
equal.sd	4	-1046.69	2101.4	94.035	< 2.2e-16 ***
equal.xd	5	-1053.16	2116.3	106.968	< 2.2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Cape MTE

Full dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	**7**	**-1114.7**	**2243.4**		
equal.d	6	-1230.7	2473.4	231.973	< 2.2e-16 ***
equal.s	5	-1119.3	2248.6	9.157	0.01027 *
equal.x	6	-1130.0	2272.1	30.687	3.033e-08 ***
equal.sxd	3	-1256.9	2519.8	284.341	< 2.2e-16 ***
equal.sx	4	-1132.2	2272.4	34.950	1.248e-07 ***
equal.sd	4	-1234.9	2477.8	240.369	< 2.2e-16 ***
equal.xd	5	-1233.5	2477.0	237.608	< 2.2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Reduced dataset:

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	**7**	**-873.47**	**1760.9**		

equal.d	6	-937.33	1886.7	127.726	< 2.2e-16 ***
equal.s	5	-875.77	1761.5	4.607	0.0999 .
equal.x	6	-886.01	1784.0	25.078	5.504e-07 ***
equal.sxd	3	-952.66	1911.3	158.382	< 2.2e-16 ***
equal.sx	4	-887.82	1783.6	28.704	2.584e-06 ***
equal.sd	4	-939.02	1886.0	131.111	< 2.2e-16 ***
equal.xd	5	-937.99	1886.0	129.032	< 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Australia MTE

Full dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	**7**	**-1115.9**	**2245.9**		
equal.d	6	-1166.7	2345.5	101.600	< 2.2e-16 ***
equal.s	5	-1131.2	2272.3	30.443	2.451e-07 ***
equal.x	6	-1133.2	2278.4	34.525	4.207e-09 ***
equal.sxd	3	-1181.9	2369.9	131.981	< 2.2e-16 ***
equal.sx	4	-1133.3	2274.6	34.714	1.400e-07 ***
equal.sd	4	-1172.3	2352.6	112.754	< 2.2e-16 ***
equal.xd	5	-1166.8	2343.6	101.714	< 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Reduced dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	**7**	**-902.01**	**1818.0**		
equal.d	6	-948.86	1909.7	93.701	< 2.2e-16 ***
equal.s	5	-912.99	1836.0	21.951	1.712e-05 ***
equal.x	6	-915.37	1842.7	26.720	2.352e-07 ***
equal.sxd	3	-964.32	1934.6	124.610	< 2.2e-16 ***
equal.sx	4	-915.38	1838.8	26.743	6.665e-06 ***
equal.sd	4	-953.22	1914.5	102.426	< 2.2e-16 ***
equal.xd	5	-948.89	1907.8	93.761	< 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

California MTE

Full dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	**7**	**-1088.0**	**2190.1**		
equal.d	6	-1173.7	2359.4	171.338	< 2.2e-16 ***
equal.s	5	-1097.8	2205.6	19.519	5.774e-05 ***
equal.x	6	-1099.5	2210.9	22.813	1.786e-06 ***
equal.sxd	3	-1202.0	2410.1	227.995	< 2.2e-16 ***
equal.sx	4	-1105.1	2218.2	34.168	1.826e-07 ***
equal.sd	4	-1181.3	2370.7	186.623	< 2.2e-16 ***
equal.xd	5	-1197.6	2405.1	219.047	< 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Reduced dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	**7**	**-879.38**	**1772.8**		
equal.d	6	-928.68	1869.4	98.596	< 2.2e-16 ***
equal.s	5	-883.02	1776.0	7.271	0.026365 *
equal.x	6	-884.35	1780.7	9.931	0.001625 **
equal.sxd	3	-949.39	1904.8	140.021	< 2.2e-16 ***
equal.sx	4	-887.28	1782.6	15.793	0.001250 **
equal.sd	4	-933.54	1875.1	108.309	< 2.2e-16 ***
equal.xd	5	-944.85	1899.7	130.938	< 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Chile MTE

Full dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	7	-1060.5	2134.9		
equal.d	6	-1070.1	2152.2	19.2789	1.129e-05 ***
equal.s	5	-1061.9	2133.8	2.8883	0.235951
equal.x	6	-1061.4	2134.8	1.8130	0.178143
equal.sxd	3	-1072.5	2150.9	23.9712	8.094e-05 ***
equal.sx	**4**	**-1063.3**	**2134.6**	**5.6638**	**0.129165**
equal.sd	4	-1070.7	2149.4	20.4337	0.000138 ***
equal.xd	5	-1070.2	2150.5	19.5288	5.746e-05 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Reduced dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	7	-844.74	1703.5		
equal.d	6	-858.68	1729.4	27.880	1.291e-07 ***
equal.s	5	-851.08	1712.2	12.686	0.001759 **
equal.x	**6**	**-845.03**	**1702.1**	**0.590**	**0.442460**
equal.sxd	3	-860.92	1727.8	32.358	1.617e-06 ***
equal.sx	4	-852.33	1712.7	15.183	0.001667 **
equal.sd	4	-859.14	1726.3	28.798	2.470e-06 ***
equal.xd	5	-858.74	1727.5	28.010	8.275e-07 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Mediterranean Basin MTE

Full dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	**7**	**-1069.2**	**2152.3**		
equal.d	6	-1106.3	2224.6	74.332	< 2.2e-16 ***
equal.s	5	-1092.1	2194.3	45.964	1.045e-10 ***
equal.x	6	-1091.1	2194.2	43.928	3.406e-11 ***
equal.sxd	3	-1113.7	2233.4	89.052	< 2.2e-16 ***
equal.sx	4	-1092.3	2192.6	46.297	4.905e-10 ***
equal.sd	4	-1107.2	2222.5	76.156	2.220e-16 ***
equal.xd	5	-1106.9	2223.8	75.499	< 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Reduced dataset

	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
full	**7**	**-874.12**	**1762.2**		
equal.d	6	-892.19	1796.4	36.136	1.840e-09 ***
equal.s	5	-877.59	1765.2	6.922	0.03139 *
equal.x	6	-876.84	1765.7	5.428	0.01982 *
equal.sxd	3	-899.26	1804.5	50.263	3.183e-10 ***
equal.sx	**4**	**-877.82**	**1763.6**	**7.383**	**0.06065 .**
equal.sd	4	-892.88	1793.8	37.512	3.586e-08 ***
equal.xd	5	-893.09	1796.2	37.925	5.817e-09 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Supporting Information 4

Phylogenetic reconstruction of the Rhamnaceae.

Granites and *Alphitonia* in the Pomaderreae appear as sister lineage to the Colletieae + *Schistocarpaea johnsonii* + the remaining Pomaderreae (pp = 0.9). As suggested by Richardson et al. (2000a), *Schistocarpaea johnsonii*, which is part of the unclassified '*incertae sedis*' group, seems to be closely related to the Colletieae and/or the Pomaderreae. Furthermore, *Ziziphus* in the Paliureae appears to be paraphyletic, confirming previously published phylogenetic results (Islam and Simmons 2006). The New World *Ziziphi* form a clade, and are sister to *Paliurus* + *Hovenia* + the Old World *Ziziphi*, although only with a pp of 0.5. Nevertheless, the sister group relationship between the Old World *Ziziphi* and *Paliurus* was strongly supported (pp = 1). *Ziziphus celata* , *Ziziphus pubescens* and *Ziziphus rivularis* are more closely related to the early diverging Rhamnaceae lineages including the Ampelziziphoid group, the Ventilagineae and the Rhamneae, confirming previous findings by Islam and Simmons (2006). Although Islam and Simmons argue that their *Ziziphus pubescens* may have been misidentified, the appearance of *Ziziphus rivularis*, which was absent in their study, in the same clade suggests that these species may actually belong here (pp = 1 for the clade including *Ziziphus pubescens*, *Z. rivularis*, *Bathiorhamnus, Doepfeldia* and *Ampeloziziphus*). *Ziziphus celata* is sister to a clade consisting of *Krugidendron, Karwinskia* and *Condalia*, however, this has low support (but pp = 0.5). Most of the species belonging to the *incertae sedis* cannot be confidently placed in the Rhamnaceae phylogeny due to very low support. *Colubrina* appears as sister to the Gouanieae (pp = 0.4), and a clade consisting of *Ceanothus*, *Emmenosperma* and *Lasiodiscus* (pp = 0.9) is sister to a clade containing Paliureae, Gouanieae and *Colubrina* (pp = 0.6).

Nevertheless, we resolved some of the deeper relationships within the Rhamnaceae, which seemed problematic in previous studies (Richardson et al. 2004). The Rhamnaceae are estimated to be ~93 My old (crown node, 95% HPD 84 – 101 Mya), and the first diverging clade separates the ampeloziziphoid groups and the rhamnoid group (83 Mya, 95% HPD 71 – 94, pp = 0.95), from the remaining Rhamnaceae (79 Mya, 95% HPD 70 – 87, pp = 1). The clade consisting of the Pomaderreae, the Colletieae and *Schistocarpaea* (93 Mya, 95% HPD 57 – 81, pp = 0.9) is sister to a clade consisting of the Gouanieae, the Paliureae, *Colubrina, Ceanothus, Lasiodiscus, Emmenosperma* and the Phyliceae (72 Mya, 95% HPD 63 – 81, pp = 0.9). Within this last clade, the Phyliceae are the first diverging lineage (31 Mya, 95% HPD 23 – 42, pp = 1), but the remaining clade consisting of Gouanieae, Paliureae, *Colubrina, Ceanothus, Lasiodiscus* and *Emmenosperma* (69 Mya, 95% HPD 61 - 78) has low support (pp = 0.55).

Table S1 Rhamnaceae species numbers, habitats and climate zones. xls table available from: **http://onlinelibrary.wiley.com/doi/10.1111/evo.12605/suppinfo**

Table S2 GenBank accession numbers.

Taxon name	*ndh*F	*mat*K	*rbc*L	ITS	*rpl*16	*psb*A	*trn*L-F
Adolphia_californica		AF049847.1		AF048973.1			
Adolphia_infesta			AJ390055.1				KP299387
Alphitonia_aff_incana_Chase_2179			AJ390049.1	AF328830.1			KP299388
Ampelozizyphus_amazonicus			AJ390037.1				AJ390341.1
Barbeya_oleoides		JF317418.1	AJ225788.1				AJ225795.1
Bathiorhamnus_cryptophorus			AJ390036.1				AJ390340.1
Berchemia_discolor		JF270655.1	AJ225786.1	AY626455.1			AJ225793.1
Berchemia_racemosa_CW07	KP299595			JN900290.1	KP299297	KP299546	KP299389
Blackallia_biloba				AY911558.1			EF528505.1
Ceanothus_americanus	U78893.1	AF049797.1		HQ325309.1			
Ceanothus_arboreus		AF049798.1		AF048902.1			
Ceanothus_caeruleus				AF328835.1			AJ225798.1
Ceanothus_confusus		AF049820.1		AF048933.1			
Ceanothus_cordulatus	U78894.1	AF049799.1	U78904.1	AF048905.1			HQ325601.1
Ceanothus_crassifolius		AF049821.1		HQ325353.1			
Ceanothus_cuneatus_var_rigidus		AF049825.1		HQ325359.1			
Ceanothus_cyaneus		AF049800.1		AF048906.1			
Ceanothus_diversifolius		AF049801.1		HQ325318.1			
Ceanothus_fendleri	U78895.1			HQ325319.1			
Ceanothus_ferrisiae		AF049827.1		HQ325362.1			
Ceanothus_foliosus_var_vineatus		AF049803.1		HQ325323.1			
Ceanothus_fresnensis		AF049828.1		HQ325363.1			
Ceanothus_gloriosus_var_gloriosus		AF049831.1		AF048944.1			
Ceanothus_greggii_var_vestitus		AF049833.1		AF048946.1			
Ceanothus_griseus		AF049804.1		AF048912.1			
Ceanothus_hearstiorum		AF049805.1		HQ325324.1			
Ceanothus_impressus		AF049806.1		HQ325326.1			
Ceanothus_incanus		AF049807.1		HQ325328.1			
Ceanothus_integerrimus	U78896.1	AF049808.1		HQ325329.1			
Ceanothus_lemmonii		AF049809.1		HQ325331.1			
Ceanothus_leucodermis		AF049810.1		HQ325332.1			
Ceanothus_maritimus		AF049834.1		HQ325369.1			
Ceanothus_masonii		AF049835.1		HQ325370.1			
Ceanothus_megacarpus _var_megacarpus		AF049838.1		HQ325372.1			
Ceanothus_oliganthus_var_sorediatus		AF049811.1		AF048923.1			
Ceanothus_ophiochilus		AF049839.1		HQ325373.1			
Ceanothus_papillosus_var_roweanus		AF049812.1		HQ325339.1			
Ceanothus_parryi		AF049813.1		HQ325340.1			
Ceanothus_parvifolius		AF049814.1		AF048927.1			

Ceanothus_pinetorum		AF049840.1		AF048959.1			
Ceanothus_prostratus	EU002210.1			AF048960.1			
Ceanothus_pumilus	U78902.1	AF049841.1	U78905.1	HQ325379.1			HQ325602.1
Ceanothus_purpureus		AF049842.1		HQ325380.1			
Ceanothus_sanguineus	U78897.1	AF049815.1	U06795.1	AF048928.1			
Ceanothus_sonomensis		AF049844.1		AF048969.1			
Ceanothus_spinosus		AF049816.1		HQ325343.1			
Ceanothus_thyrsiflorus	U78898.1	AF049817.1	U59827.1	HQ325345.1			
Ceanothus_tomentosus		AF049818.1		HQ325347.1			
Ceanothus_velutinus	U78899.1			HQ325349.1			
Ceanothus_verrocosus		AF049846.1		HQ325383.1			
Colletia_hystrix					KP299298		AY460409.1
Colletia_paradoxa_CW1052					KP299299		KP299390
Colletia_spinosa_CW12	KP299596			KP299471	KP299300		KP299391
Colletia_ulicina			U59819.1		KP299301		AJ390364.1
Colubrina_asiatica		GU135023.1	AJ390047.1	AF328831.1		GU135352.2	AJ390350.1
Colubrina_reclinata			AJ390065.1	AF328832.1			AJ390370.1
Condalia_microphylla			AJ390032.1	AY626456.1			AJ390334.1
Crumenaria_erecta			AJ390042.1	HQ325385.1			AJ390346.1
Cryptandra_alpina				AY911540.1			EF528488.1
Cryptandra_amara				AY911545.1			EF528489.1
Cryptandra_arbutiflora				AY911546.1			EF528491.1
Cryptandra_connata				AY911561.1			EF528503.1
Cryptandra_dielsii				AY911553.1			EF528500.1
Cryptandra_ericoides				AY911541.1			EF528487.1
Cryptandra_gemmata				AY911547.1			EF528494.1
Cryptandra_hispidula				AY911542.1			EF528492.1
Cryptandra_intratropica				AY911549.1			EF528495.1
Cryptandra_lanosiflora				AY911543.1			EF528490.1
Cryptandra_micrantha				AY911544.1			EF528493.1
Cryptandra_mutila				AY911552.1			EF528498.1
Cryptandra_myriantha			AJ390060.1	AY911552.1			AJ390360.1
Cryptandra_nola				AY911548.1			EF528496.1
Cryptandra_pungens				AY911551.1			EF528497.1
Dirachma_socotrana		JF317423.1	AJ225789.1				AJ225796.1
Discaria_chacaye			AF307911.1		KP299302		AJ225797.1
Discaria_toumatou			AF307912.1				AY642150.1
Doerpfeldia_cubensis			AJ390038.1				AJ390342.1
Elaeagnus_angustifolia			U17038.1				DQ838727.1
Elaeagnus_bockii		JF317425.1	JF317484.1				
Elaeagnus_pungens		GU135102.1	GU135269.1				
Elaeagnus_umbellata		AY257529.1	HM849968.1				HM769678.1
Emmenosperma_alphitonioides			AJ390048.1	HQ340159.1			AJ390351.1
Frangula_alnus_subsp_baetica				AY626450.1			AY626429.1

Frangula_azorica		HM850914.1	HM850010.1				
Frangula_betulifolia				AY626445.1			AY626424.1
Frangula_californica		AF288121.1		AY626442.1			AF348565.1
Frangula_purshiana	U78903.1			AY626430.1			AY626411.1
Frangula_rupestris_CW14	KP299597					KP299547	KP299392
Gouania_mauritiana	JF317447.1	JF317427.1	AJ390040.1				AJ390344.1
Granitites_intangendus				HQ340160.1			AJ306540.1
Helinus_integrifolius		JF270816.1	AJ390043.1	HQ325386.1			AJ390347.1
Hippophae_neurocarpa_subsp_neurocarpa		JF954049.1	JF941944.1				HM769680.1
Hippophae_rhamnoides_subsp_gyantsensis		JF954038.1	JF941925.1				EU099999.1
Hippophae_rhamnoides_subsp_sinensis		JF317428.1	JF941935.1				JQ289181.1
Hippophae_rhamnoides_subsp_yunnanensis		JF954040.1	JF941931.1				JQ289187.1
Hippophae_salicifolia		JF954054.1	U59821.1				HM769681.1
Hippophae_tibetana		JF954062.1	JF941950.1				HM769674.1
Hovenia_acerba	KP299598	HQ415396.1	HQ415232.1		KP299303	HQ415578.1	KP299393
Hovenia_dulcis	KP299599		AJ390039.1	DQ146607.1	KP299304		KP299394
Hovenia_trichocarpa	KP299600	JF317429.1	HQ427241.1	DQ146608.1		HQ427084.1	DQ146565.1
Karwinskia_humboldtiana			AJ390031.1				
Krugiodendron_ferreum			AJ390028.1				AJ390331.1
Lasiodiscus_mildbraedii			AJ390050.1	AF328833.1			AJ390353.1
Maesopsis_eminii			AJ390034.1			KC667854.1	AJ390336.1
Nesiota_elliptica			AJ225783.1	AF328823.1			KP299395
Noltea_africana			AJ390054.1	AF328822.1			KC633945.1
Paliurus_hemsleyanus				DQ146609.1			DQ146567.1
Paliurus_ramosissimus				DQ146612.1			DQ146568.1
Paliurus_spina_christi	KP299601		AJ390051.1	DQ146613.1	KP299305	EU075112.1	AJ390354.1
Papistylus_grandiflorus				AY911559.1			EF528504.1
Phylica_acmaephylla_SH173	KP299602			KP299472	KP299306	KP299548	KP299396
Phylica_aemula				KP299473	KP299307	KP299549	KP299397
Phylica_alba_SH142	KP299603			KP299474	KP299308	KP299550	KP299398
Phylica_altigena_SH201	KP299604			KP299475	KP299309		KP299399
Phylica_ambigua_SH119	KP299605			KP299476	KP299310		KP299400
Phylica_apiculata_SH167	KP299606				KP299311		KP299401
Phylica_arborea		GQ248177.1	GQ248666.1	AF328801.1		GQ248363.1	AF327603.1
Phylica_atrata_SH41	KP299607			KP299477	KP299312	KP299551	KP299402
Phylica_axillaris							
Phylica_axillaris_SH147	KP299608			KP299478	KP299313		KP299403
Phylica_buxifolia	KP299609			AF328813.1	KP299314		AF327614.1
Phylica_callosa_SH102	KP299610				KP299315	KP299552	KP299404
Phylica_cephalantha_SH155	KP299611			KP299479			KP299405
Phylica_confusa_SH141	KP299612			KP299480	KP299316		KP299406
Phylica_constricta	KP299613			KP299481	KP299317	KP299553	KP299407

Phylica_cryptandroides_SH117	KP299614			KP299482	KP299318		KP299408
Phylica_curvifolia_SH25	KP299615			KP299483	KP299319	KP299554	
Phylica_cuspidata	KP299616			KP299484	KP299320		KP299409
Phylica_cylindrica_SH120	KP299617			KP299485			
Phylica_debilis_debilis_SH30				KP299486	KP299321	KP299555	KP299410
Phylica_debilis_fourcadei_SH13				KP299487	KP299322		KP299411
Phylica_dioica_SH47	KP299618			KP299488	KP299323		KP299412
Phylica_diosmoides_SH79	KP299619			KP299489	KP299324	KP299556	KP299413
Phylica_disticha_SH194	KP299620				KP299325		
Phylica_dodii_SH92	KP299621						KP299414
Phylica_elsieae_SH51	KP299622			KP299490	KP299326	KP299557	
Phylica_emirnensis_DUB1305				KP299491	KP299327	KP299558	KP299415
Phylica_empetroides_SH80	KP299623			KP299492	KP299328	KP299559	KP299416
Phylica_ericoides_SH165	KP299624			KP299493	KP299329		KP299417
Phylica_excelsa_SH57				KP299494	KP299330	KP299560	KP299418
Phylica_floribunda_SH182	KP299625				KP299331		
Phylica_fourcadei_SH16				KP299495	KP299332		KP299419
Phylica_fruticosa_SH200	KP299626			KP299496	KP299333	KP299561	KP299420
Phylica_gnidioides_SH9				KP299497	KP299334	KP299562	KP299421
Phylica_gracilis_SH105	KP299627			KP299498	KP299335		KP299422
Phylica_harveyi_SH197	KP299628				KP299336		
Phylica_humilis_SH212	KP299629			KP299499	KP299337	KP299563	
Phylica_imberbis_SH36	KP299630			KP299500	KP299338	KP299564	KP299423
Phylica_incurvata_SH185	KP299631			KP299501	KP299339	KP299565	KP299424
Phylica_intrusa_SH62	KP299632			KP299502	KP299340		KP299425
Phylica_karroica_SH18				KP299503	KP299341		KP299426
Phylica_keetii_mollis_SH14				KP299504		KP299566	KP299427
Phylica_laevigata_SH188	KP299633			KP299505		KP299567	KP299428
Phylica_laevis_SH83	KP299634			KP299506		KP299568	KP299429
Phylica_lanata_SH135	KP299635			KP299507	KP299342	KP299569	KP299430
Phylica_lasiantha	KP299636			KP299508	KP299343	KP299570	KP299431
Phylica_lasiocarpa_SH40	KP299637			KP299509	KP299344	KP299571	KP299432
Phylica_leipoldtii_SH203	KP299638			KP299510	KP299345		KP299433
Phylica_levynsiae_SH206				KP299511	KP299346	KP299572	KP299434
Phylica_litoralis_SH10				KP299512	KP299347	KP299573	KP299435
Phylica_lucens_SH88	KP299639			KP299513	KP299348	KP299574	KP299436
Phylica_meyerii_SH20				KP299514	KP299349	KP299575	KP299437
Phylica_minutiflora_SH94	KP299640				KP299350		
Phylica_montana				AF328811.1			AF327612.1
Phylica_nigrita_SH38	KP299641			KP299515	KP299351	KP299576	KP299438
Phylica_nitida	KP299642		AJ390053.1	AF328821.1	KP299352		KP299439
Phylica_obtusifolia_SH61	KP299643			KP299516	KP299353		KP299440
Phylica_odorata_SH66	KP299644			KP299517	KP299354	KP299577	KP299441

Phylica_oleaefolia	KP299645				KP299355		
Phylica_oleifolia				AF328812.1			
Phylica_paniculata			GQ248667.1	AF328808.1	KP299356	GQ248364.1	AF327606.1
Phylica_parviflora_SH156	KP299646			KP299518	KP299357	KP299578	KP299442
Phylica_parvula_SH187	KP299647			KP299519	KP299358	KP299579	KP299443
Phylica_pauciflora_SH205	KP299648			KP299520	KP299359		KP299444
Phylica_pinea_SH26	KP299649			KP299521	KP299360		KP299445
Phylica_plumigera				AF328818.1			AF327618.1
Phylica_plumosa_SH110	KP299650			KP299522	KP299361		KP299446
Phylica_polifolia			AJ225784.1	AF328805.1			AJ390373.1
Phylica_propinqua_SH152	KP299651			KP299523	KP299362		
Phylica_pubescens				AF328814.1			Y16771.1
Phylica_pulchella_SH204	KP299652			KP299524	KP299363		KP299447
Phylica_purpurea_pearsonii_SH33	KP299653			KP299525	KP299364	KP299580	KP299448
Phylica_purpurea_purpurea_SH153	KP299654			KP299526	KP299365		KP299449
Phylica_pustulata_SH113	KP299655			KP299527	KP299366		KP299450
Phylica_rigida_SH65	KP299656			KP299528	KP299367		KP299451
Phylica_rogersii_SH168	KP299657			KP299529	KP299368	KP299581	KP299452
Phylica_rubra_SH180	KP299658			KP299530	KP299369		KP299453
Phylica_selaginoides_SH164	KP299659			KP299531	KP299370		KP299454
Phylica_sericea_SH138	KP299660			KP299532	KP299371		KP299455
Phylica_sp_SH133				KP299533		KP299582	KP299456
Phylica_spicata				AF328816.1			AF327616.1
Phylica_stenantha_SH56	KP299661			KP299534	KP299372	KP299583	KP299457
Phylica_strigosa_SH49	KP299662			KP299535	KP299373	KP299584	KP299458
Phylica_thodei	KP299663			AF328810.1	KP299374		AF327611.1
Phylica_thunbergiana_SH96	KP299664				KP299375	KP299585	KP299459
Phylica_trachyphylla_MP529	KP299665			KP299536	KP299376		KP299460
Phylica_villosa_SH118	KP299666			KP299537	KP299377		KP299461
Phylica_virgata_SH53	KP299667			KP299538	KP299378	KP299586	
Phylica_vulgaris_major_SH59	KP299668			KP299539	KP299379	KP299587	KP299462
Phylica_vulgaris_vulgaris_SH131	KP299669			KP299540	KP299380		KP299463
Phylica_wittebergensis_SH130				KP299541	KP299381	KP299588	KP299464
Pleuranthodes_hillebrandii			AJ390045.1				KP299465
Polianthion_wichurae				AY911554.1			EF528501.1
Pomaderris_angustifolia				AY911568.1			EF528518.1
Pomaderris_brevifolia				AY911564.1			EF528513.1
Pomaderris_elliptica				AY911570.1			EF528519.1
Pomaderris_forrestiana				AY911566.1			EF528514.1
Pomaderris_grandis				AY911567.1			EF528512.1
Pomaderris_obcordata				AY911563.1			EF528516.1
Pomaderris_oraria				AY911571.1			EF528515.1
Pomaderris_phylicifolia							EF528520.1

Pomaderris_phylicifolia_var_ericoides				AY911573.1			
Pomaderris_prunifolia_subsp_edgerleyi				AY911574.1			EF528521.1
Pomaderris_rotundifolia				AY911565.1			EF528550.1
Pomaderris_rugosa			AJ390063.1	AF328826.1			AJ390363.1
Pomaderris_tropica				AY911569.1			EF528517.1
Reissekia_smilacina			AJ390041.1	DQ146614.1			AJ390345.1
Retanilla_trinervia			AJ390056.1				AY642154.1
Reynosia_uncinata			AJ390029.1				AJ390339.1
Rhamnella_franguloides			AJ390027.1	AY626454.1			AJ390330.1
Rhamnidium_elaeocarpum			AJ390030.1	AY626452.1			AJ390332.1
Rhamnus_alaternus	KP299670			AY626435.1	KP299382	KP299589	AY626416.1
Rhamnus_alnifolia		EU749367.1	EU676975.1			EU750517.1	
Rhamnus_alpina				AY626438.1			AY626417.1
Rhamnus_cathartica		AY257533.1	L13189.2	AY626436.1		EU750518.1	
Rhamnus_crenata		HQ427385.1	HQ427242.1	AY626443.1		HQ427085.1	AY626422.1
Rhamnus_crenulata				AY626448.1			AY626428.1
Rhamnus_crocea				AY626434.1			AY626415.1
Rhamnus_davurica	AF130225.1			AY626441.1			AY626420.1
Rhamnus_esquirolii				AY626440.1			AY626419.1
Rhamnus_fallax_CW01	KP299671			KP299542	KP299383		KP299466
Rhamnus_glandulosa				AY626446.1			AY626425.1
Rhamnus_lycioides			AJ390070.1	AY626437.1		KP299590	AJ390374.1
Rhamnus_prinoides			AM235104.1	AY626432.1			AY626413.1
Rhamnus_pumila	KP299672			AY626433.1	KP299384		AY626414.1
Rhamnus_purpurea				AY626439.1			AY626418.1
Rhamnus_purshianus_CW10	KP299673			KP299543		KP299591	KP299467
Rhamnus_saxatilis	KP299674			AY626447.1		KP299592	AY626426.1
Rhamnus_sphaerospermus_CW03	KP299675					KP299593	KP299468
Rhamnus_staddo				AY626449.1			AY626427.1
Rhamnus_taquetii_CW02	KP299676			KP299544	KP299385	KP299594	KP299469
Rhamnus_utilis		JF317432.1	JF317492.1				
Sageretia_thea			AJ225785.1	AY626453.1			AJ225792.1
Schistocarpaea_johnsonii			AJ390046.1	AY911539.1			AJ390349.1
Scutia_buxifolia			AJ390033.1	AY626451.1			AJ390335.1
Shepherdia_argentea			AJ225787.1				AJ390372.1
Shepherdia_canadensis			U17039.1				GQ245525.1
Siegfriedia_darwinioides			AJ390064.1	AF328827.1			EF528507.1
Spyridium_burragorang				AY911594.1			EF528536.1
Spyridium_buxifolium				AY911595.1			EF528508.1
Spyridium_cordatum				AY911538.1			EF528530.1
Spyridium_eriocephalum				AY911581.1			EF528522.1
Spyridium_globulosum			AJ390058.1	AF328828.1			EF528529.1
Spyridium_gunnii				AY911593.1			EF528524.1
Spyridium_halmaturinum				AY911582.1			EF528527.1

Spyridium_mucronatum				AY911589.1			EF528528.1
Spyridium_nitidum				AY911584.1			EF528531.1
Spyridium_parvifolium		AF049849.1		AF048975.1			EF528526.1
Spyridium_scortechinii				AY911596.1			EF528537.1
Spyridium_subochreatum				AY911585.1			EF528532.1
Spyridium_thymifolium				AY911586.1			EF528533.1
Spyridium_ulicinum				AY911592.1			EF528523.1
Stenanthemum_centrale				AY911605.1			EF528544.1
Stenanthemum_complicatum			AJ390059.1	AY911599.1			EF528539.1
Stenanthemum_gracilipes				AY911560.1			EF528506.1
Stenanthemum_humile				AY911600.1			EF528540.1
Stenanthemum_leucophractum				AY911604.1			EF528545.1
Stenanthemum_petraeum				AY911601.1			EF528541.1
Stenanthemum_pomaderroides			AJ390057.1				AJ251690.1
Stenanthemum_reissekii				AY911603.1			EF528543.1
Trichocephalus_stipularis			AM235105.1	KP299545	KP299386		KP299470
Trymalium_angustifolium				AY911580.1			EF528548.1
Trymalium_elachophyllum				AY911576.1			EF528547.1
Trymalium_floribundum			AJ390062.1	AY911578.1			AJ390362.1
Trymalium_ledifolium			AJ390061.1	AF328829.1			EF528551.1
Trymalium_minutiflorum				AY911557.1			
Trymalium_monospermum				AY911577.1			EF528546.1
Trymalium_wayi				AY911562.1			EF528509.1
Ventilago_viminalis			AJ390035.1				AJ390337.1
Ziziphus_acidojujuba				DQ146572.1		EU075108.1	DQ146528.1
Ziziphus_amole			HQ325595.1	DQ146579.1			DQ146535.1
Ziziphus_apetala				EU075094.1		EU075103.1	
Ziziphus_attopensis				EU075099.1		EU075104.1	
Ziziphus_calophylla			HQ325597.1	DQ146580.1			DQ146536.1
Ziziphus_celata				DQ146581.1			DQ146538.1
Ziziphus_fungii				EU075095.1		EU075102.1	
Ziziphus_glabrata				DQ146583.1			AJ225799.1
Ziziphus_guatemalensis				DQ146584.1			DQ146541.1
Ziziphus_horsfieldii				DQ146586.1			DQ146542.1
Ziziphus_incurva				EU075096.1		EU075111.1	
Ziziphus_jujuba_var_spinosa			GQ436668.1	JF421556.1			
Ziziphus_lotus				DQ146587.1		HE602473.1	DQ146543.1
Ziziphus_mairei				EU075092.1		EU075107.1	
Ziziphus_mauritiana			HQ325598.1	DQ146589.1		EU075110.1	DQ146545.1
Ziziphus_mistol				DQ146590.1			DQ146547.1
Ziziphus_montana				EU075091.1			
Ziziphus_mucronata				DQ146592.1			DQ146549.1
Ziziphus_obtusifolia_var_canescens				DQ146595.1			DQ146552.1
Ziziphus_oenoplia				DQ146598.1			AB235097.1
Ziziphus_ornata			AJ390052.1				AJ390355.1

Ziziphus_pubescens				DQ146600.1			DQ146555.1
Ziziphus_rivularis		JF271008.1	JF265667.1				
Ziziphus_rugosa			HQ325599.1	DQ146601.1		EU075105.1	DQ146557.1
Ziziphus_spina_christi			HQ325596.1	DQ146604.1			DQ146558.1
Ziziphus_taylori				DQ146605.1			DQ146561.1
Ziziphus_thyrsiflora				DQ146606.1			DQ146562.1
Ziziphus_xiangchengensis				EU075090.1		EU075106.1	

Table S3 Comparison of median node ages for Rhamnaceae clades under different scenarios of missing data in the alignment, resulting from BEAST (all same settings, see main text).

Clade	**59% missing data, 308 taxa 7 markers (Original)**	**0% missing data, 207 taxa 2 markers**	**44% missing data, 152 taxa 7 markers (each taxon at least 3)**	**36% missing data, 101 taxa 7 markers (each taxon at least 4)**	**27% missing data, 47 taxa 7 markers (each taxon at least 5)**
Rhamnaceae crown	92.6	91.4	92.4	94	85.1
Rhamneae + *Ziziphus sp.*	83.1	83.8			
Rhamneae + *Measopsis*	54		60		
Rhamneae	46.4	48.5	48.4	51.7	42.7
Berchemia	32.0	32.2	38.6	37.6	
Scutia + *Sageretia*	26.6	23.2	30.8		
Berchemia + *Scutia* + *Sageretia*	38.1	37.5	39.2		
Rhamnidium + *Rhamnella* + *Condalia* + *Ziziphus celata*	33.3	33.3	30.6		
Frangula	8.5	6.4	8.2		
Rhamnus	23.5	22.7	24.1	23.8	
Rhamnus + *Frangula*	30.8	31.5	30.9	34.4	
All excluding Rhamneae	78.6	79.5	80.7	77.8	
Ziziphea	66.3		68.4		
Reissekia + *Crumenaria*	34.1	40.2	35.2		
Helinus + *Gouania*	38.9		43.1	45.1	
Reissekia + *Crumenaria* + *Helinus* (Gouanieae)	50.5	64.6	55.1		
Ziziphus clade 1	29.0	28.7			
Ziziphus clade 2	38.6	33.6	33.6		
Ziziphus clade 2 + *Paliurus*	45.8	46.1	45.8	45.7	
Ziziphus clade 2 + *Paliurus* + *Hovenia*	57.4		60.5	60.3	
Paliurus	21.9	21.0			
Hovenia	15.2	17.2	14.5	16.3	14.5
Colubrina	38.4	39.3	37.9		
Alphitonia + *Granitites* + Pomaderreae + *Schistocarpeae* + Colletieae	69.5	70.1			
Colletia	10.8		6.3		
Colletia + *Discaria* (+ *Adolphia*)	23.3		22.2		

Alphitonia + *Granitites*	34.2	27.4			
Pomaderreae + *Schistocarpeae*	62.7	46.5			
Pomaderreae	34.3	32.3	30.9		
Pomaderris + *Siegfriedia* + *Trymalium*	27.4	27.8			
Pomaderris + *Siegfriedia* + *Trymalium* + *Spyridium*	29.8		27.1		
Trymalium	15.7	14.7	15.6		
Pomaderris + *Siegfriedia*	20.6	19.5	17.7		
Pomaderris	15.9	14.7			
Stenenthemum + *Serichonus* + *Papistylus* + *Blackallia*	30.9	28.6			
Serichonus + *Papistylus* + *Blackallia*	24.4	23.1			
Papistylus + *Blackallia*	13.1	12.7			
Stenanthemum	26.0	23.7			
Spyridium	15.8	15.5			
Cryptandra	22.5	21.2			
Ceanothus + *Emmenosperma*	48.6	38.3	43.5		
Ceanothus	24.4	23.0	25.2	27.29	21.6
Ceanothus clade 2	12.2		12.2	12.3	
Phyliceae	31.1	27.5	31.2		
Phylica + *Trichocephalus*	26.0			27.4	
Phylica	23.0	20.5	23.1	24.8	17.2

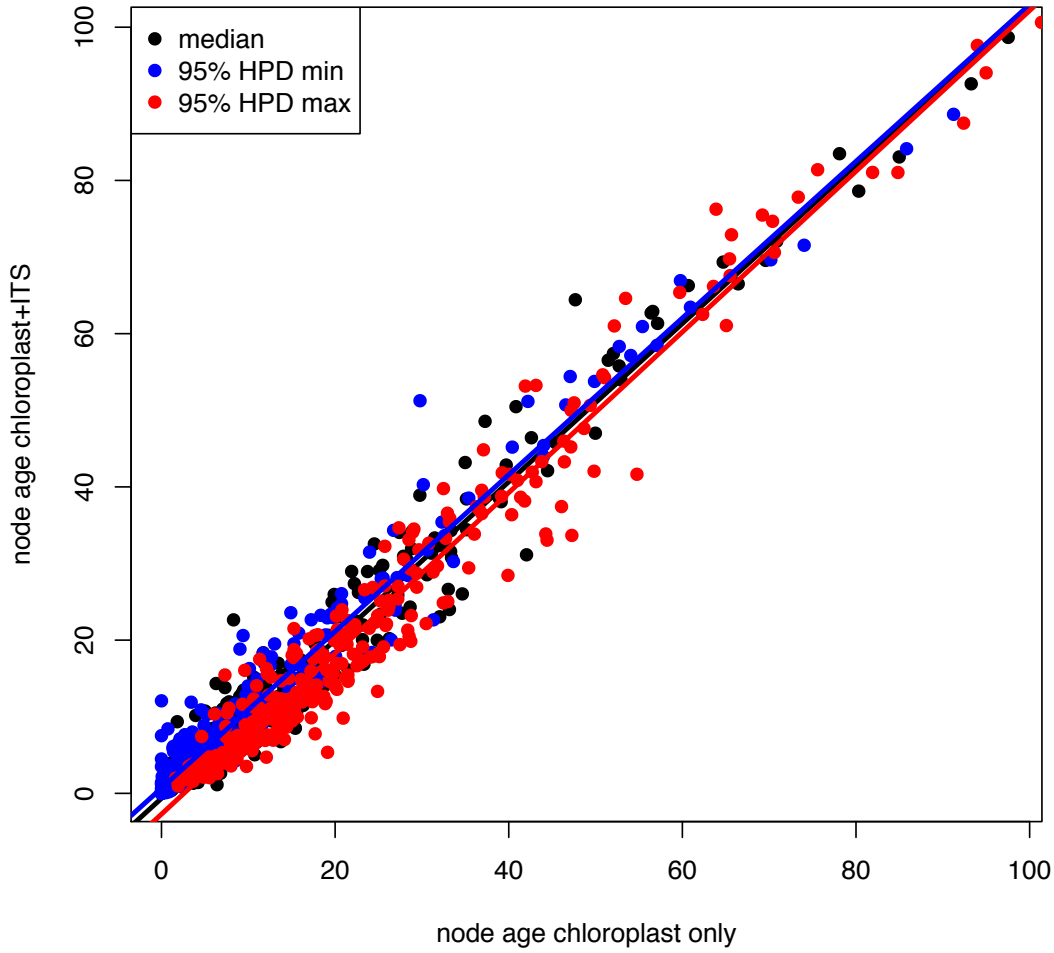
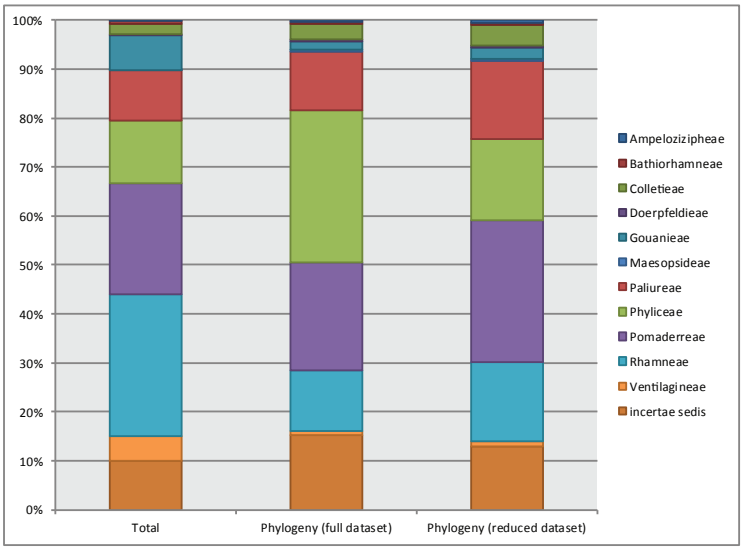


Figure S1: Rhamnaceae median node ages and the minimum and maximum bound of the 95% Highest Posterior Density of the estimated age of the combined dataset (ITS + chloroplast) against the chloroplast only dataset. A significant correlation with a R^2 of 0.96 for median node ages was detected.



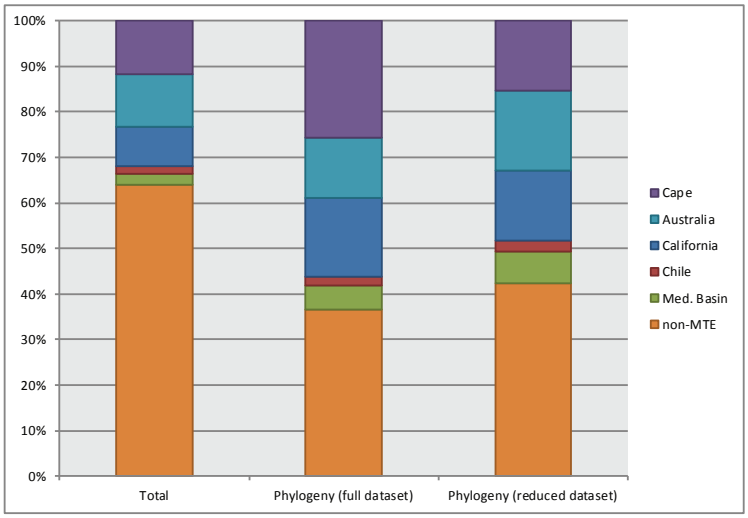


Figure S2: Sampling proportions of Rhamnaceae tribes and MTEs in the full dataset (phylogeny with 280 species) and the reduced dataset (phylogeny with 214 species) compared to the total (1055 species).

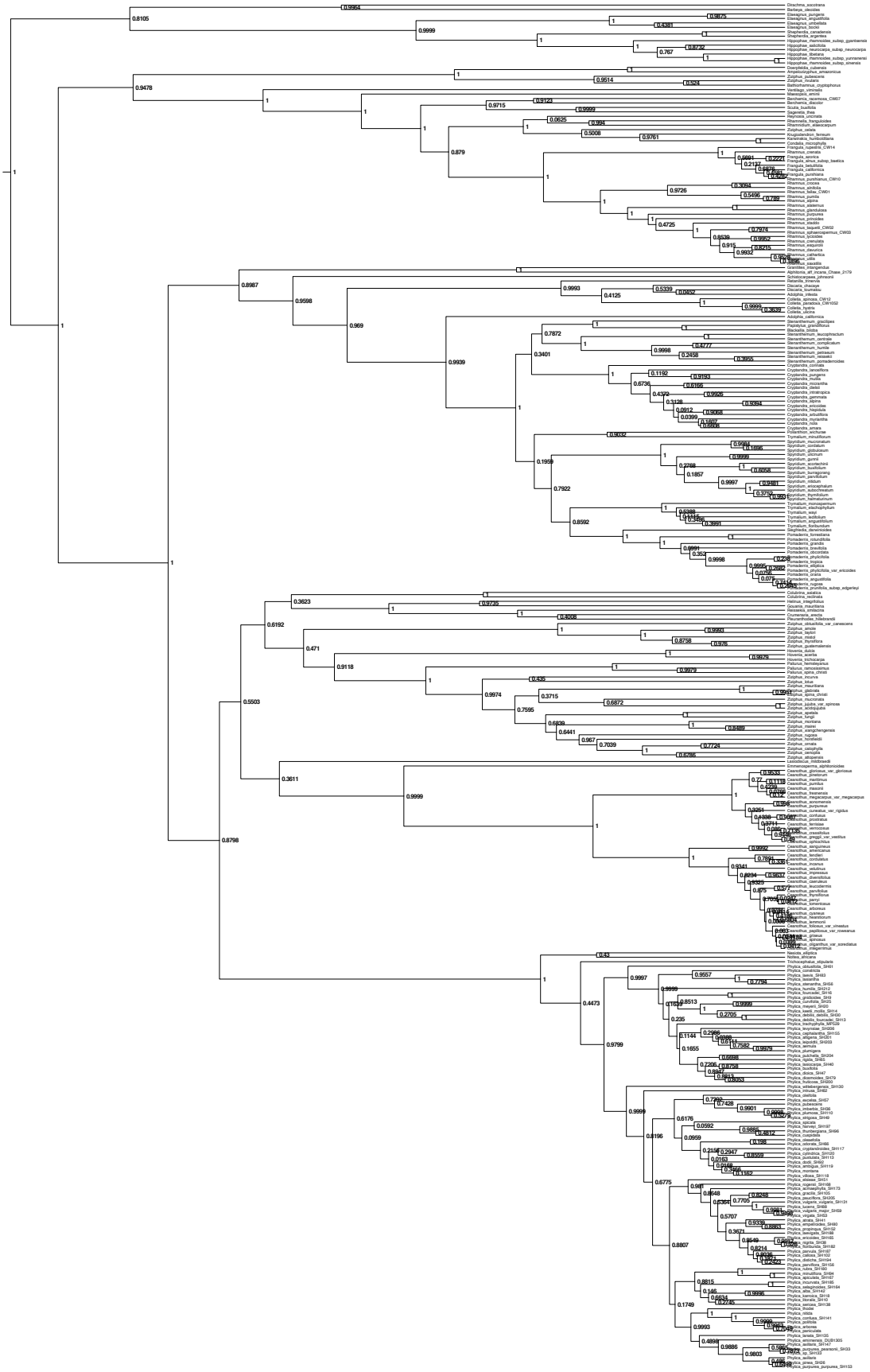


Figure S3: Rhamnaceae MCC tree resulting from the BEAST analysis including all taxa; Posterior Probabilities (p.p.) are shown on the nodes.

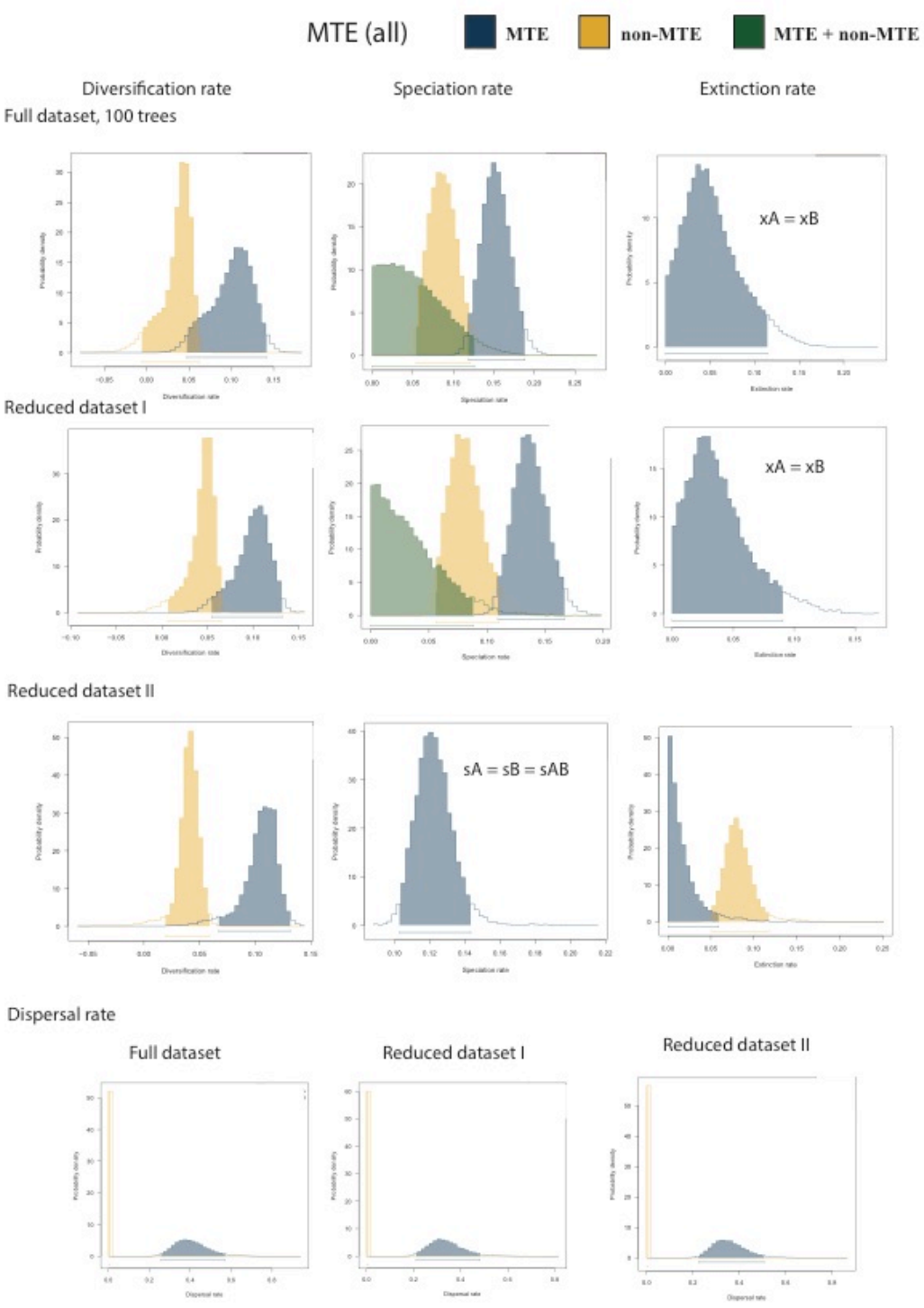
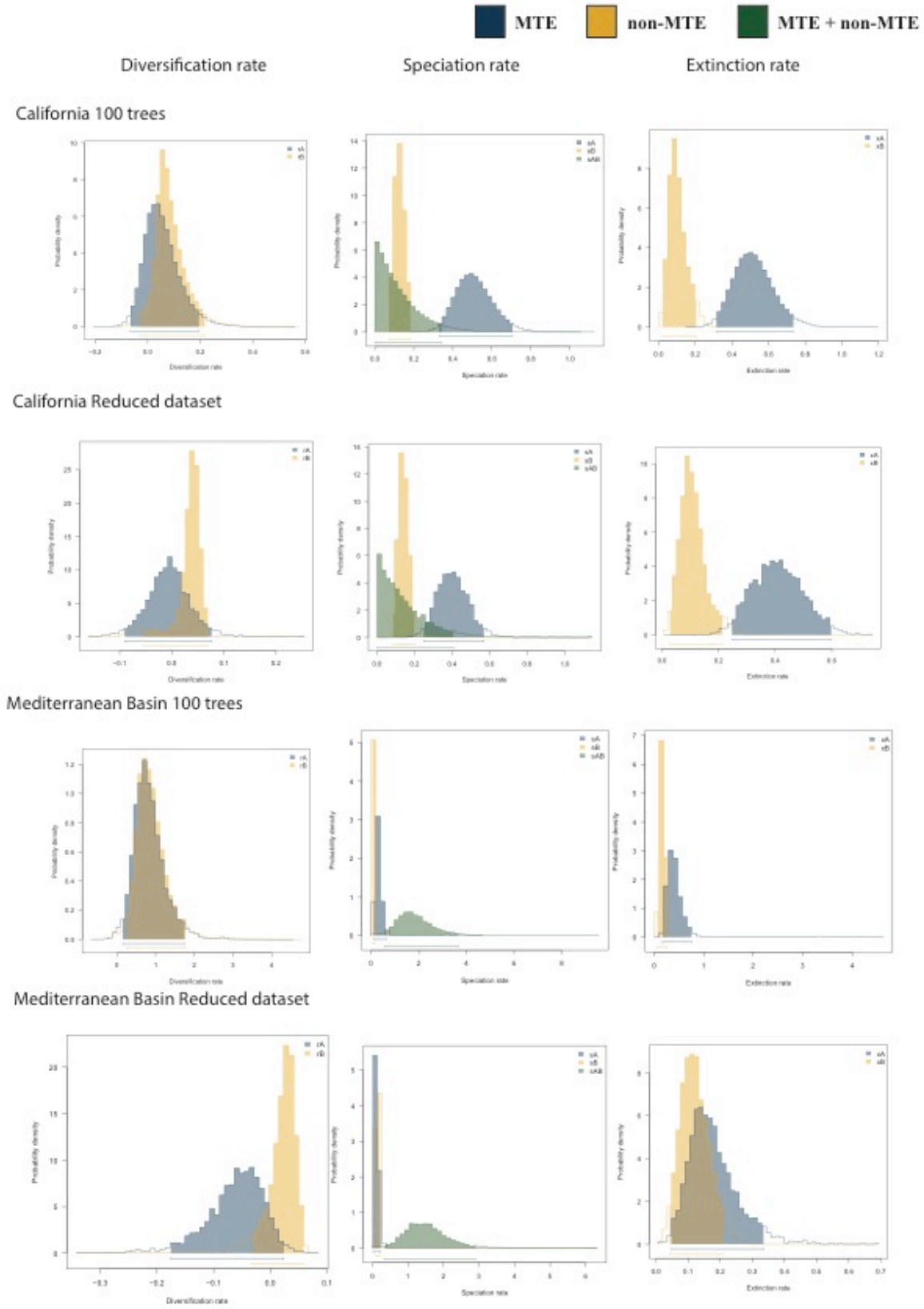
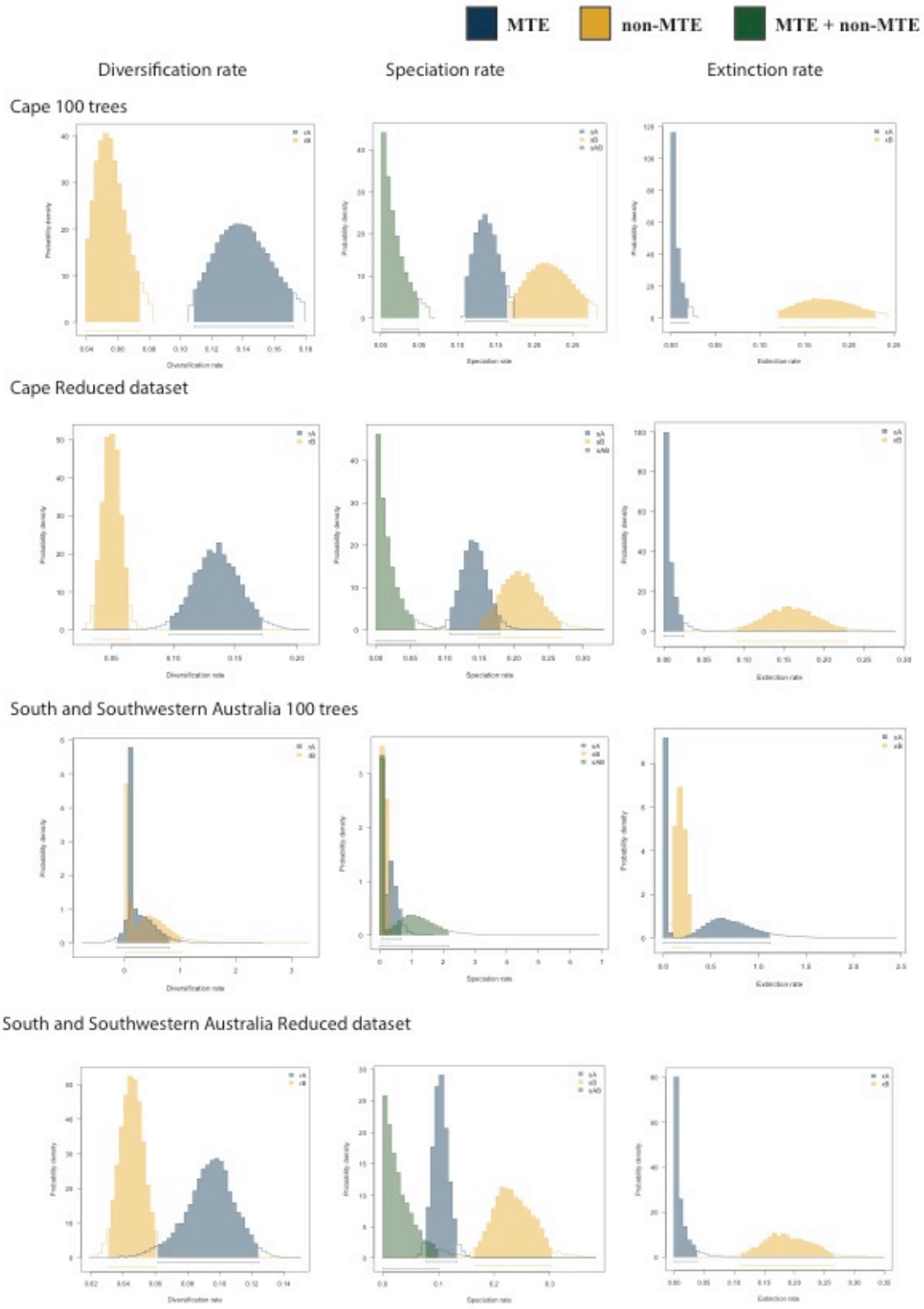


Figure S4: Posterior distributions of the GeoSSE analysis estimating speciation and extinction rates for Rhamnaceae MTE-lineages (all MTEs combined) against non-MTE lineages over 100 trees, and for the reduced datasets (I = extinction rate constrained, II = speciation rate constrained). MTE-lineages have higher diversification rates, due to either high speciation rates or due to low extinction rates. The dispersal rates for the different analyses are also shown, suggesting that dispersal rates from MTEs to elsewhere are higher than the other way around.


MTE
non-MTE
MTE + non-MTE
Diversification rate
Speciation rate
Extinction rate
California 100 trees
California Reduced dataset
Mediterranean Basin 100 trees
Mediterranean Basin Reduced dataset


MTE
non-MTE
MTE + non-MTE
Diversification rate
Speciation rate
Extinction rate
Cape 100 trees
Cape Reduced dataset
South and Southwestern Australia 100 trees
South and Southwestern Australia Reduced dataset

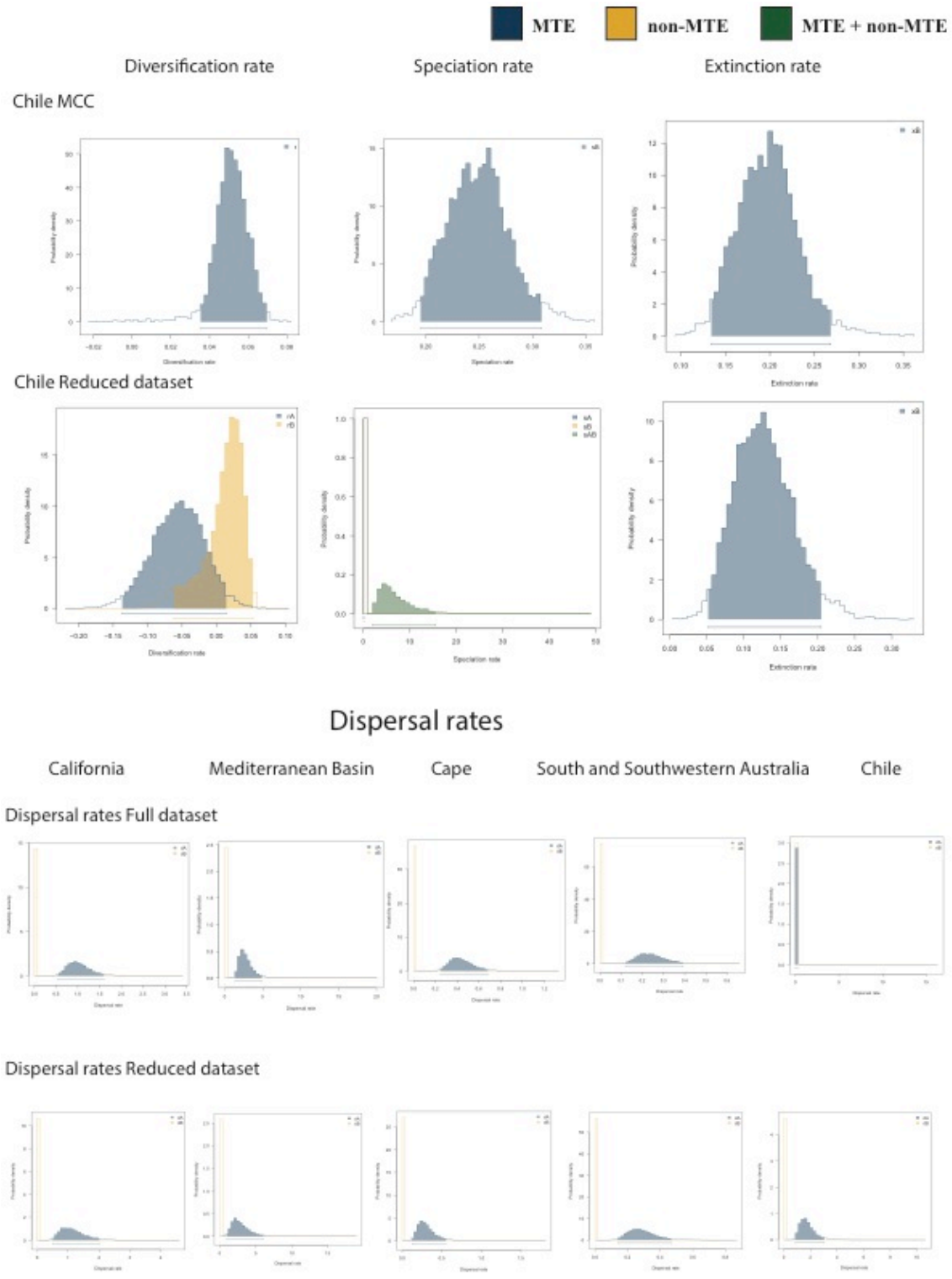


Figure S5: Posterior distributions of the GeoSSE analysis estimating net diversification, speciation, extinction and dispersal rates for MTEs of California, the Mediterranean Basin, the Cape, Western Australia and Chile against the remaining Rhamnaceae over 100 trees, and for the reduced datasets (following model selection, see Supporting Information 3).

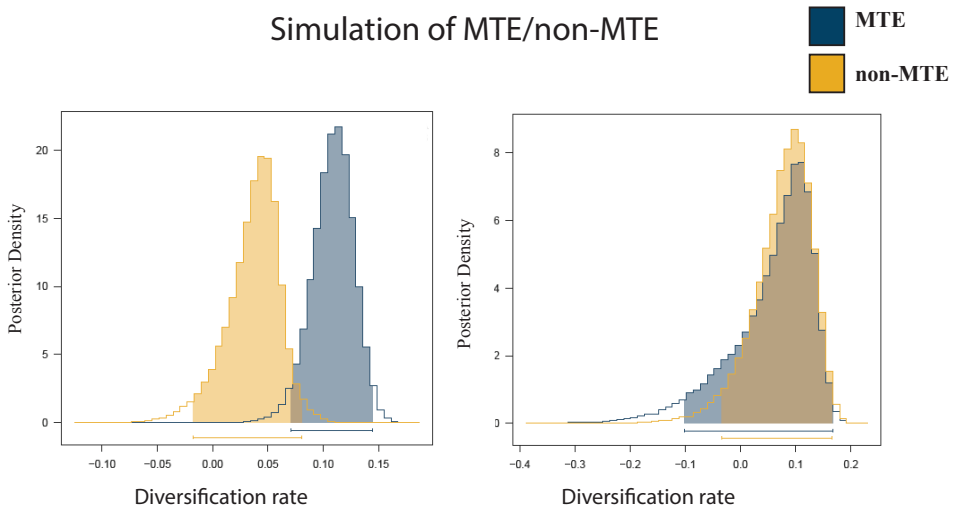


Figure S6: Posterior distributions of the net diversification rate resulting from 100 simulated trees with known speciation and extinction rates (unequal between states in A (left), equal in B (right)), after pruning the tree with states proportionally to obtain our observed proportion of MTE and non-MTE lineages.

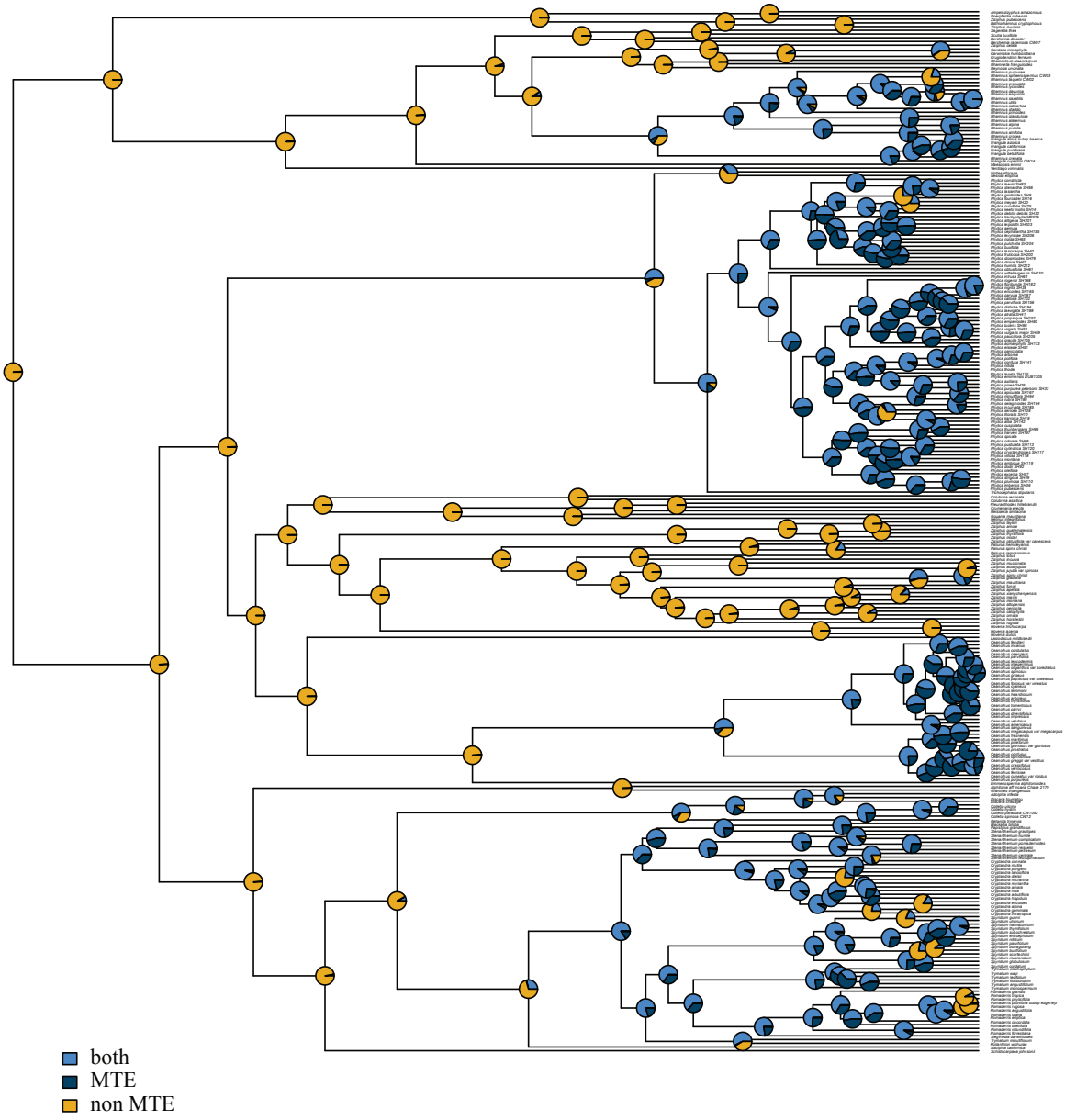


Figure S7: GeoSSE ancestral area reconstruction (states: MTE/non MTE/both) on the Rhamnaceae MCC tree resulting from the BEAST analysis.

CHAPTER IV: BEYOND CLIMATE: CONVERGENCE IN FAST EVOLVING SCLEROPHYLLS IN CAPE AND AUSTRALIAN RHAMNACEAE PREDATES THE MEDITERRANEAN CLIMATE

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Author contributions:

REO and HPL designed the research; REO collected functional trait data and performed principal component analyses, ancestral state reconstructions, evolutionary model testing, tests for correlated evolution and diversification rate analyses; REO wrote the manuscript, with comments from HPL.

Abstract

Morphological convergence in lineages found in the five Mediterranean-type ecosystems (MTEs) of the world has long been interpreted as adaptation to climatic similarities among these regions. Here, we challenge this model using the globally distributed family Rhamnaceae. We show that functional trait data (specific leaf area, leaf size, spinescence, leaf phenology, growth form and leaf margin type) tends to three optima, which correspond to (a) the edaphically specialized Cape and Australian regions, (b) the Mediterranean type climates, but edaphically normal Chile, California and Mediterranean basin, and (c) the non-Mediterranean type habitats. We find that Rhamnaceae in California, Chile and the Mediterranean Basin are predominantly characterized by non-sclerophylly, which is the ancestral state in Rhamnaceae, and Rhamnaceae in the Cape and Australian MTEs by sclerophylly. We show that these leaf character syndromes have evolved prior to summer-drought climates in MTEs, thus showing that they cannot be interpreted as adaptations to this selective regime. However, sclerophylly evolved contemporaneously with the transitions to Cape and Australian MTEs, and may therefore potentially be an adaptation to edaphic conditions typical of these regions. Furthermore, our results suggest that the evolution of sclerophylly has contributed to increased diversification rates of Phyliceae and Pomaderreae in respectively the Cape and Australia by reducing extinction rates, and thereby facilitating evolutionary persistence. The historical relatively stable conditions in the Cape and Australia are consistent with this persistence hypothesis. This lowered extinction rate may thus account for not only the ecological, but also the floristic dominance of sclerophylly in the Cape and Australian MTEs.

Keywords:

Diversification rate, sclerophylly, character syndrome, Cape flora, kwongan, extinction rate.

Introduction

Convergence and climate

Macro-climate is thought to drive trait assembly, resulting in geographically separated analogous biomes and vegetation types (Schimper 1903). This may be the result of convergent evolution. Convergent evolution is the evolution of similar traits in independent evolutionary lineages, but repeated convergence to the same morphology may have different causes. It is generally accepted that convergence is the result of selection on traits by particular environments, and these traits can consequently be regarded as 'adaptations'. However, convergence can also arise under conditions other than natural selection, for example when traits evolve in response to selective regimes but may confer high fitness in other environments ('exaptations' to those other environments, Gould and Vrba 1982), or as a genetic and / or physiological correlated response to selection on other traits (Losos 2011). The striking morphological convergence of lineages in the five Mediterranean-type ecosystems (MTEs) has often been linked to adaptations to similarities in climate (Schimper 1903, Cody and Mooney 1978). However, the hypothesis that this convergence is adaptive to climate has not been critically tested (but see Ackerly 2004a, Ackerly 2009). Furthermore, the macro-evolutionary consequences of these morphological changes in terms of lineage diversification remain enigmatic.

Although geographically separated on different continents, the five Mediterranean-type ecosystems (MTEs) of the world (the southern African Cape, California-Baja California, the Mediterranean Basin, central Chile and South and Southwest Australia) are characterized by hot, dry summers and cool, wet winters (Aschmann 1973, Castri 1973, Kottek et al. 2006) (hereafter

'Mediterranean climate'). These comparable climatic conditions in MTEs may have selected for plants with similar functional traits, resulting in analogous vegetation types such as dry shrub- or heathland (Schimper 1903, Specht 1979, Cowling et al. 1996). This vegetation type is called 'fynbos' in the southern African Cape (hereafter Cape), 'chaparral' in California-Baja California (hereafter California), 'kwongan' in South and Southwest Australia (hereafter Australia), 'maquis' in the Mediterranean Basin and 'matorral' in central Chile (hereafter Chile). However, the geomorphological history and long-term climatic stability is very different among the MTEs (Cowling et al. 2014). Furthermore, they differ in timing of the onset of the Mediterranean climate: in the Pliocene (2 – 5 Million years ago [Ma]) in the Mediterranean Basin and California (Axelrod 1973, Suc 1984, Suc and Popescu 2005), in the mid- to late-Miocene (10 – 15 Ma) in the Cape region (Cowling et al. 2009, Dupont et al. 2011) and similarly in Chile (8 – 15 Ma) (Armesto et al. 2007) and from the early Miocene onwards (20 Ma) in Western Australia (Hopper and Gioia 2004, Martin 2006). Thus, the climatic similarities belie complex geomorphological and historical differences, which suggest that two groups of MTEs can be defined. The Cape and Australia (CA), characterized by oligotrophic soils and long-term climatic stability, and California, Chile and the Mediterranean Basin (CCM) characterized by more eutrophic soils and a more dynamic Quaternary climate. We therefore challenge the idea that comparable extant climates among the MTEs have provided the selective regime that led to trait convergence.

Mediterranean character syndromes

Two character syndromes were described for MTE floras, although these syndromes may be primarily indicative of the floras of the Mediterranean Basin, California and Chile (Herrera 1992, Verdú et al. 2003). The first syndrome includes sclerophyllous, evergreen leaves and small, unisexual greenish or brownish flowers with a reduced perianth and large seeds dispersed by animals ('sclerophyllous syndrome' hereafter). Examples of genera with the sclerophyllous-syndrome are *Olea* (Oleaceae) and *Pistacia* (Anacardiaceae) (Herrera 1992). The second syndrome includes the alternative character states of non-sclerophyllous, deciduous leaves and larger hermaphroditic non-greenish or brownish flowers and smaller seeds dispersed by agents other than animals ('non-sclerophyllous syndrome' hereafter, *sensu* Verdú and Pausas (2013)). Examples of genera with the non-sclerophyllous syndrome are *Cistus* (Cistaceae) and *Rosmarinus* (Lamiaceae) (Herrera 1992). Specht (1969) showed how the relative contribution of these syndromes seems to be dependent on soil fertility as well as climate when comparing sclerophyllous vegetation characteristics between the Mediterranean Basin, California and Southern Australia. Cowling and Witkowski (1994) compared trait convergence in edaphically matched sites between the Cape and Australia, and found predominantly convergence of sclerophyllous shrublands, with commonality of growth forms, leaf size and leaf consistency, although leaf spines were more common in Australia. The mix of non-sclerophyllous and sclerophyllous character syndromes in MTEs suggests that this pattern of convergence in physiognomy in MTEs may be more complex than previously thought.

The non-sclerophyllous syndrome has been hypothesized to be 'adaptive' to the Mediterranean-climate, whereas the sclerophyllous syndrome could be 'exaptive' to this climate regime (Herrera 1992, Verdú et al. 2003, Ackerly 2004a, Sniderman et al. 2013). Traits are called 'adaptations' if they are naturally selected for by the environment and 'exaptations' (Gould and Vrba 1982) if they have previously evolved by natural selection for a particular function, but are coopted for a new use (Gould and Vrba 1982). An example of an exaptation are feathers, which initially evolved for heat regulation, were coopted for display, and later coopted for use in bird flight (Gould and Vrba 1982). Generally, the 'exaptive' sclerophyllous syndrome seemed to have evolved in the pre-Mediterranean (sub)-tropical ancestors of Mediterranean lineages, and was ecologically 'filtered' into the Californian and Mediterranean Basin MTEs when these arose in the Plio-Pleistocene. In

contrast, the non-sclerophyllous syndrome was derived or selected for after the onset of the Mediterranean climate (Herrera 1992, Verdú et al. 2003, Ackerly 2004a, Sniderman et al. 2013).

Functionality of Mediterranean character syndromes

Sclerophyllous leaves are associated with a relatively low photosynthetic capacity, a high proportion of leaf stored carbon, low leaf-nitrogen concentrations and a low ratio between leaf area and mass (low specific leaf area, SLA) (Wright et al. 2004). These traits can provide an advantage under water-stress (i.e. summer-drought) conditions, herbivory-stress and / or nutrient-poor conditions (Fonseca et al. 2000, Wright et al. 2004). Although water-stress in summer is a general feature of MTEs, the degree of nutrient-deficiency is highly variable between MTEs. The Cape and Australia are characterized by soils developed from nutrient-poor, very old parent material or from Quaternary infertile siliceous sands. These acidic soils have been heavily weathered and highly leached. In the Mediterranean Basin large areas of calcium-rich/high-pH soils have probably resulted from direct or indirect human impacts on the original forests over the past 3000 to 4000 years, and in California, moderately and strongly leached soils may be found, but only weakly leached soils are found in central Chile (Specht and Moll 1983, Rambal 2001). These differences between the Cape/Australia and California/Chile/Mediterranean Basin may have selected for species with different degrees of sclerophylly, resulting in variation in sclerophyllous/non-sclerophyllous character syndromes between MTEs.

Diversification in MTEs

Mediterranean-type ecosystems are exceptional in their species-richness and endemism, possibly a product of increased net diversification rates (speciation rate - extinction rate) (Crayn et al. 2006, Sauquet et al. 2009, Lancaster and Kay 2013, Onstein et al. 2015) and / or more time to accumulate diversity (Valente et al. 2011, Cowling et al. 2014). The persistence of lineages in environments, for example through low extinction rates, and therefore high net diversification rates, may be affected by the morphological and physiological traits of lineages, which can affect fitness through their effects on growth and survival (Violle et al. 2007). Indeed, Verdu and Pausas (2013) showed increased rates of diversification of lineages with the non-sclerophyllous syndrome in the Mediterranean Basin after the onset of the Mediterranean climate ca. 3.6 Ma, and Onstein et al. (2014) showed increased rates of diversification after correlated shifts to low SLA and small leaves (sclerophylly) and fynbos habitats in three Cape clades. This suggests that both these Mediterranean character syndromes under the 'right' conditions may lead to increased diversification.

Rhamnaceae as a study system

Previous studies on Mediterranean character syndromes have focussed primarily on the MTEs in California, Chile and the Mediterranean Basin (Herrera 1992, Verdú et al. 2003, Ackerly 2004a, Ackerly 2009, Verdú and Pausas 2013), consequently the evolution of these syndromes and their effect on diversification rates in the Cape and Australia is largely unknown. Furthermore, most of these studies have focused on ecological communities rather than on clades of related species (but see Ackerly 2004a). Combining all species present in a community into a phylogenetic tree may lead to misleading conclusions with respect to timing of evolution of character syndromes and estimates of diversification rates, due to biased sampling and absence of related non-Mediterranean species and their traits.

In this study we therefore investigate character syndrome evolution and the effects on diversification rates in a single, large clade: the Buckthorn family, Rhamnaceae Juss. (Rosales) (ca. 1055 species, Onstein et al. 2015 and references therein). This family is globally distributed, occurred ancestrally (most likely) in tropical rainforest biomes and colonised all five MTEs in independent dispersal events (Onstein et al. 2015). Rhamnaceae consists of predominantly warm-temperate woody

shrubs, with insect-pollinated flowers and a vegetative morphology ranging from spiny desert-shrubs to large tropical forest trees or lianas, and foliage ranging from aphyllous to entire, evergreen leaves, and from leaves with revolute margins to toothed deciduous leaves. Rhamnaceae has biotically-dispersed fleshy fruits or nuts, although some species have non-fleshy, mostly wind-dispersed fruits. Representing both the sclerophyllous and non-sclerophyllous character syndromes, and characterized by at least five independent colonization events of the five MTEs, Rhamnaceae may be a good clade in which to investigate convergence and non-convergence (divergence) of these syndromes and their effects on diversification rates in MTEs.

Hypotheses

If there is convergence among the MTE Rhamnaceae, then there should be more than one selective optimum in the evolution of the leaf traits. Furthermore, if convergence is driven by the Mediterranean climate, all MTEs should have the same optimum, whereas if edaphic factors are important then the CA and CCM regions should have different optima. We test whether the shift from the ancestral condition to these optima is adaptive or exaptive, by comparing scenarios of correlated evolution and the age of the MTE-shift to the age of evolution of these traits. Finally, we explore whether the evolution of the dominance of species with these traits could be the macro-evolutionary consequence of an increased diversification rate of lineages bearing these traits.

Our results indicate two Rhamnaceae character syndromes for the MTEs: relatively large, sometimes deciduous, non-sclerophyllous, toothed leaves, and presence of spines in California, Chile and the Mediterranean Basin and the opposite character syndrome of small, sclerophyllous, evergreen leaves with entire margins in the Cape and Australia. There has been correlated evolution between CA and CCM and their corresponding character syndromes, and in all cases the character syndromes evolved prior to the onset of the Mediterranean climate, supporting the hypothesis of exaptation rather than adaptation to the Mediterranean climate. The presence of two character syndromes suggests, instead, that these particular syndromes may be adaptations to factors such as soil nutrient-deficiency in the current MTE-areas. Diversification rates in CA have been particularly rapid, and the sclerophyllous character syndrome may potentially have contributed to this by causing persistence through low extinction rates of Rhamnaceae lineages in these MTEs.

Materials and Methods

Functional trait and climate sampling

In order to assign species to Mediterranean and other biomes, we downloaded 197,681 occurrence data-points for 784 Rhamnaceae species (74% of total) obtained from the Global Biodiversity Information Facility, accessed in May 2013 (http://www.gbif.org/). These were linked to the Köppen-Geiger climate classification of the world (Kottek et al. 2006). If species occurred in climate zones ‘Csa’, ‘Csb’ and/or ‘Csc’ they were assigned to the Mediterranean climate, in climate zone ‘Af’ to the tropical rainforest climate, in climate zones ‘BWk’ and/or ‘BWh’ to the desert climate, and in climate zone ‘Cfb’ to the temperate climate. If there were >29 occurrence data points for a species, it obtained the state of absent from these climate zones if none of the occurrence points were found in any of these zones (i.e. state ‘other’). All species with <30 data points obtained the state of ‘unknown’. In addition we obtained information on the vegetation structure a species primarily occurs in (open/closed) for 704 species (67%) from floras, monographs and webpages (data will be available from the Dryad Digital Repository). We combined these two types of information to assign species to biomes: Mediterranean climates and open vegetation to Mediterranean shrublands, tropical rainforest climates and closed vegetation to tropical rainforests, desert climates and open vegetation to deserts, and temperate climates and closed vegetation to temperate forests. We checked these assignments with information from floras and monographs for each species. This resulted in data for 592 species

for presence/absence in Mediterranean shrublands (56%), for 818 species for presence/absence in tropical rainforests (78%), for 314 species for presence/absence in deserts (30%) and for 818 species for presence/absence in temperate forests (78%).

We derived the species' character syndromes from seven binary and two continuous functional traits. These traits were selected firstly because they show variation in Rhamnaceae, secondly because they could represent possible aptations (i.e. exaptations or adaptations) to Mediterranean shrublands (Herrera 1992, Verdú and Pausas 2013), thirdly because they are easily accessible from floras, monographs and online databases and finally because they can be measured from herbarium specimens. These traits were: tree >10 meters for 884 species (presence/absence for 84%), climbing growth form for 933 species (presence/absence for 88%), deciduous leaves for 778 species (presence/absence for 74%), aphyllous for 948 species (presence/absence for 90%), spinescence for 929 species (presence/absence for 88%), toothed leaf margin for 873 species (presence/absence for 83%), revolute margins for 856 species (presence/absence for 81%), leaf area for 253 species (24%) and SLA for 232 species (22%). Leaf area was measured as the area (mm^2) of the upper side of a leaf (excluding petiole); SLA (mm^2/mg) was measured by dividing the area of the leaf (including petiole) by the weight of the leaf after drying it for 72 hours in the oven at 60 °C, following protocols by Cornelissen et al. (2003). Species means for leaf area and SLA were calculated from ten leaves taken from herbarium specimens, all taken from different individuals if available. In the case of leptophyllous leaves (<25 mm^2), we took 10–30 leaves per specimen and treated it as one leaf, due to size- and weight-errors associated with very small and light leaves. This was repeated for five specimens. We supplemented our data with leaf area and SLA data from Ackerly et al. (2004a). All data will be available from the Dryad Digital Repository.

As most of the phylogenetic comparative methods we use in this study need binary data (but see the section on selective trait optima), we defined binary states for SLA and leaf area by performing an ANOVA between biomes and traits, and evaluating which of our selected biomes deviated most from the mean in other biomes ('other') (fig. A1). To meet the requirements of the model (i.e. model residuals normally distributed, homoscedasticity), SLA and leaf area were log transformed. Based on the model parameters from the ANOVA we defined binary states for the traits, considering the average linear increase/decrease and standard deviation of these traits in MTEs, if these were significantly different from the intercept (average in other biomes). Log (SLA) < 0.6938 mm^2/mg and log (leaf area) < 1.6637 mm^2 were significantly associated with MTEs.

Character syndromes

A non-metric multidimensional scaling (NMDS) (Kruskal 1964), implemented in isoMDS from the MASS library in R (R Development Core Team 2008), was performed to evaluate the disparity of traits and character syndromes in MTEs. This approach is based on Herrera (1992) and Verdu et al. (2003). This ordination technique can deal with binary data and finds the best solution based on the specified number of dimensions, by reducing the 'stress' in the data. Ordinations were carried out on all Rhamnaceae species for which data for all seven binary traits was available (190 species). The maximum correlation with corresponding functional trait variables was plotted with envfit from the vegan library (Dixon and Dixon 2003) onto the NMDS. We assigned species to one of the two character syndromes based on their scores on component 1 and 2 in the NMDS (for details see results)

Ancestral character syndrome

We estimated the ancestral character syndrome in Rhamnaceae, and when the derived character syndrome evolved, on the Maximum Clade Credibility (MCC) tree and a set of 100 dated phylogenetic trees from the posterior distribution for 280 Rhamnaceae species (27% of total) (Onstein et al. 2015). These trees resulted from Bayesian phylogenetic analyses in BEAST 1.7.5 (Drummond

and Rambaut 2007), in which topology and divergence times were estimated using six chloroplast markers and ITS, and including eight fossil calibrations, under an uncorrelated lognormal relaxed clock model (for details see Onstein et al. 2015), the MCC tree will be deposited in the Dryad Digital Repository. We used the 'multistate' function in BayesTraits v.2 (Pagel and Meade 2006) and defined nodes for which ancestral state reconstructions had to be performed with the command 'addMRCA' followed by the list of species descending from this node. These species do not necessarily have to form a clade in all 100 trees, and inferred probabilities for character states therefore take topological uncertainty into account. We used maximum likelihood as well as Bayesian statistics to perform the ancestral state reconstructions. For the Bayesian MCMC we used a reversible jump hyper prior with an exponential prior between 0 and 100 for 10×10^6 iterations, with a burnin of 10×10^5 iterations. The reversible jump integrates results over the model space, and automatically selects viable models and parameters. The ratedev was estimated automatically, but we checked that it was between 0.2 and 0.4. For the character syndromes (non-sclerophyllous *versus* sclerophyllous), an unequal rate model was used, in which transition rates between states can vary, but for the MTEs (California, Cape, Australia, Chile, Mediterranean Basin and non-MTE) an symmetric rate model was used to reduce the number of estimated parameters, as the analysis failed to run when parameters were allowed to be estimated freely. In the symmetric rate model the back-and-forth transition rates between states are the same, resulting in 15 instead of 30 transition rates.

Correlated evolution

We tested for correlated evolution between occurring in CCM and having the corresponding non-sclerophyllous character syndrome, and between occurring in CA and having the sclerophyllous character syndrome, in BayesTraits v2 (Pagel and Meade 2006). We calculated Bayes Factors (BFs) of a model where the evolution between character syndrome and MTEs is independent (uncorrelated) to a model where they are dependent (correlated). Support for the dependent model indicates that the transition from one character state to the other in the first character (e.g. CA *versus* non-CA) is not independent on the state the second trait is in (sclerophyllous *versus* non-sclerophyllous character syndrome). We tested the following hypotheses: H0) there is no support for correlated evolution; BFs of the dependent and independent model are not significantly different, or BFs support a model of independent over dependent evolution *versus* H1) there is support for correlated evolution; BFs support a model of dependent over independent evolution.

We ran the analyses for each MTE group (presence/absence in CA *versus* non-CA and presence/absence in CCM *versus* non-CCM) and the corresponding character syndrome (presence/absence of sclerophyllous *versus* non-sclerophyllous character syndrome, respectively) separately. We first ran a maximum likelihood analysis on 100 trees. To test the accuracy of the maximum likelihood results, we ran a Bayesian MCMC for six parallel analyses for the dependent and the independent model on 100 post-burnin trees for 5×10^6 iterations and discarded a burnin of 5×10^5 iterations. We used a reversible jump hyper prior with an exponential prior between 0 and 100. The mean harmonic mean of the six runs for the dependent and independent model was calculated, and the significance of the correlation was tested by calculating the log (BF):

Log BF = 2(log [harmonic mean (dependent model)] – log [harmonic mean (independent model)]

A Log BF > 2 indicates positive evidence, > 5 strong evidence and > 10 very strong evidence for correlated evolution.

To test the hypothesis of exaptation *versus* adaptation, we evaluated transition rates of the dependent model if this model was preferred over the independent model. If the rate of gain of sclerophyllous traits inside CA is greater than elsewhere (formally written as q34 > q12, table 1), this supports the adaptation hypothesis. The same applies to if the rate of gain of non-sclerophyllous traits is greater in CCM than elsewhere. Furthermore, if the rate of transition into MTEs is higher when the

character syndrome is present than when absent (formally written as q24 > q13, table 1), this supports the exaptation hypothesis. To this end we calculated Bayes Factors of the full, unconstrained model and a model in which we constrained these transition rates to be equal (i.e. q34 = q12 or q24 = q13). Statistical support for the unconstrained over the constrained models would indicate that transition rates are significantly different, supporting the hypothesis of adaptation or exaptation.

Selective trait optima

We used the 'OUwie' R package (Beaulieu et al. 2012) to model the evolution of sclerophyllous and non-sclerophyllous character syndromes, and test for an association with MTEs. These likelihood models for continuous traits use species level data at the tips of the phylogeny and estimate parameters to best reflect the evolutionary history of the trait given the phylogeny. Models increase in complexity, from a model reflecting simple drift (Brownian motion) to Ornstein-Uhlenbeck models with several selective optima, including a parameter estimating the strength of attraction towards the optimum (α) and a parameter for the rate of stochastic evolution away from the optimum (σ^2). We tested three alternative models: (i) a neutral Brownian model (BM) of evolution of SLA, leaf area and component 1 and 2 in the NMDS, in which there is no association between MTEs and traits; (ii) a Ornstein–Uhlenbeck model, OU1 with a single optimum for each trait, which assumes that traits evolve non-neutrally but all species are pulled towards a single optimal value (θ) of the trait; and (iii) a model (OU3) with three optima for the traits (θ non-MTE, θ CA and θ CCM), which assumes that non-MTE, CA and CCM lineages have different optimal trait values. We recorded the likelihood of each model and used the Akaike Information Criterion (AIC) to identify the model that best described our data.

Effects of Mediterranean character syndrome and MTEs on diversification rates

To test for the interactive effects of character syndromes in the particular MTEs on speciation and extinction rates, we used the Multiple State Speciation and Extinction model (MuSSE multistate) (FitzJohn 2012). The MuSSE multistate model can be used to investigate the individual effects of two characters (i.e. presence of the sclerophyllous or non-sclerophyllous character syndrome and presence in CA or CCM) on speciation, extinction and transition rates, which is done by fitting a linear model. For the speciation rate, for example, the model is estimated using the parameters lambda0 (lambda of both characters in state 0) and adding the effects of both characters in state 1 additively. An interaction term (when both characters are in state 1) will indicate whether these traits may not add additively to the speciation rate, but interact in either a positive way (i.e. both characters in state 1 increase the speciation rate) or a negative way (i.e. both characters in state 1 decrease the speciation rate). The same can be done for the extinction rate. Model selection can be performed by comparing the likelihood of models with different sets of parameters with a likelihood-ratio test. In total we compared 12 different models (tables A1 and A2).

Our phylogenetic sampling contained distribution information for 263 species (102 species in CA, 64 in CCM and 97 non-MTE), and trait syndrome information for 160 species (111 sclerophyllous species, 49 non-sclerophyllous species). We therefore ran two MuSSE multistate models, for CA and CCM with the corresponding character syndrome, to test if these extrinsic and intrinsic variables affect speciation and extinction rates in an additive fashion, or if there is a positive or negative interaction between them. The preferred model was subsequently run on a set of 100 post-burnin BEAST topologies to investigate if results were sensitive to topological and branch-length variation.

Results

Character syndromes

Two clusters can be distinguished in the NMDS, separated by the diagonal (fig. 1): those species with the non-sclerophyllous syndrome, and those with the sclerophyllous syndrome. Consequently, if a species' score on component 1 < component 2, it was assigned the sclerophyllous syndrome, if its score on component 1 > component 2, it was assigned the non-sclerophyllous syndrome (fig. 1). Species with the sclerophyllous syndrome are characterized by one or more of these traits: small, evergreen leaves, a low SLA, revolute and entire margins, and absence of spines. These traits are imperfectly correlated, resulting in the spread of species in the NMDS. Species with the non-sclerophyllous syndrome are characterized by one or more of the opposite traits. Mediterranean shrublands of the Cape and Australia (CA) are characterized by species with the sclerophyllous syndrome, whereas species in the Mediterranean shrublands of California, Chile and the Mediterranean Basin (CCM) generally show the non-sclerophyllous syndrome, with the exception of the Californian *Ceanothus*, which shows a mix of non-sclerophyllous and sclerophyllous character syndromes (fig. 2).

Ancestral state reconstructions

The ancestral Rhamnaceae most probably had the non-sclerophyllous character syndrome (fig. 2). Based on these reconstructions, the sclerophyllous syndrome evolved at least five times. The ancestral geographical distribution of Rhamnaceae is less clear, and is dependent on model settings (transition rates), and whether Maximum Likelihood or Bayesian MCMC was used (fig. 2, fig. A2). However, the ancestral area was most probably non-MTE, and colonization of the five MTEs happened independently.

Correlated evolution

We found very strong support for correlated evolution for occurring in CA and CCM and having the corresponding sclerophyllous and non-sclerophyllous character syndromes (table A3). Transition rates resulting from the dependent (correlated) model (table 1) support adaptation as well as exaptation for the sclerophyllous character syndrome in CA, but these results were not supported by BFs (table 2). However, we detected significant support from BFs for exaptation of the non-sclerophyllous character syndrome to CCM (tables 2 and 3).

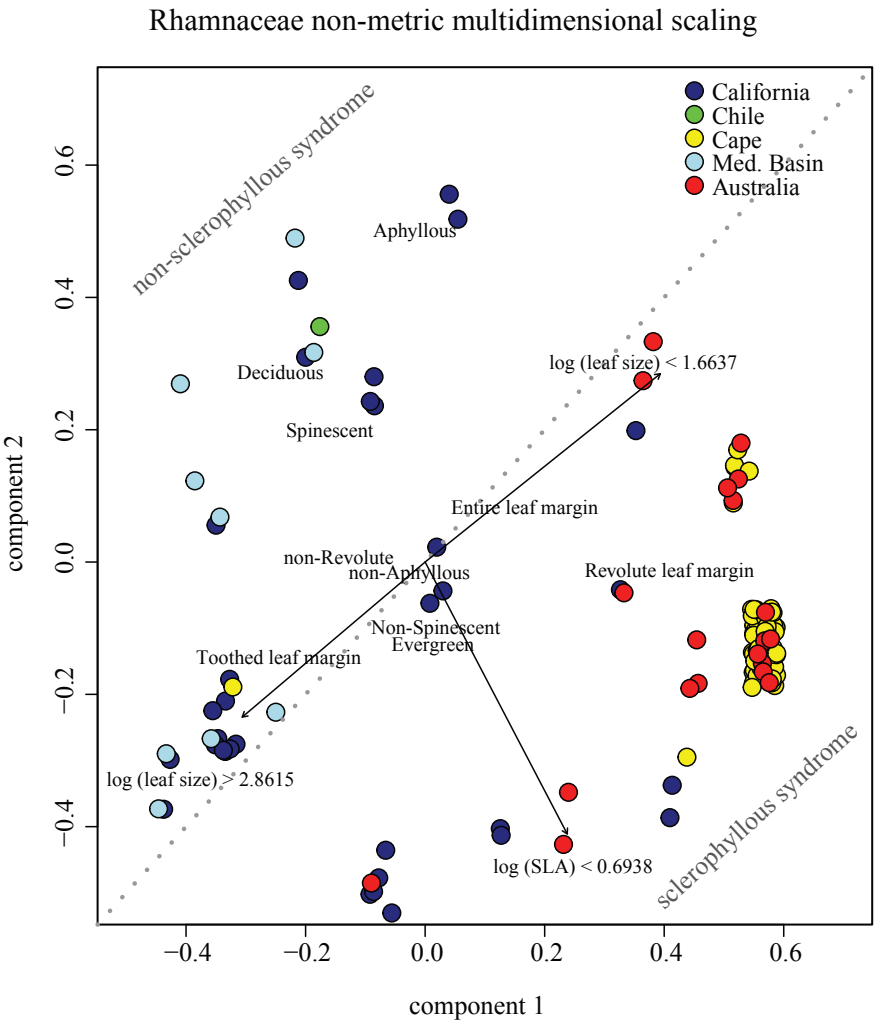

Figure 1
NMDS for 190 Rhamnaceae species. Species are coloured by MTE. Traits and their correlation in the NMDS are shown, and arrows indicate traits significantly contributing to the pattern. The diagonal (dashed line) distinguishes character syndromes of species with low SLA, small, evergreen leaves, with entire, revolute margins and absence of spines in Australia and the Cape, from the opposite character syndrome in Chile, the Mediterranean Basin and most Californian species.

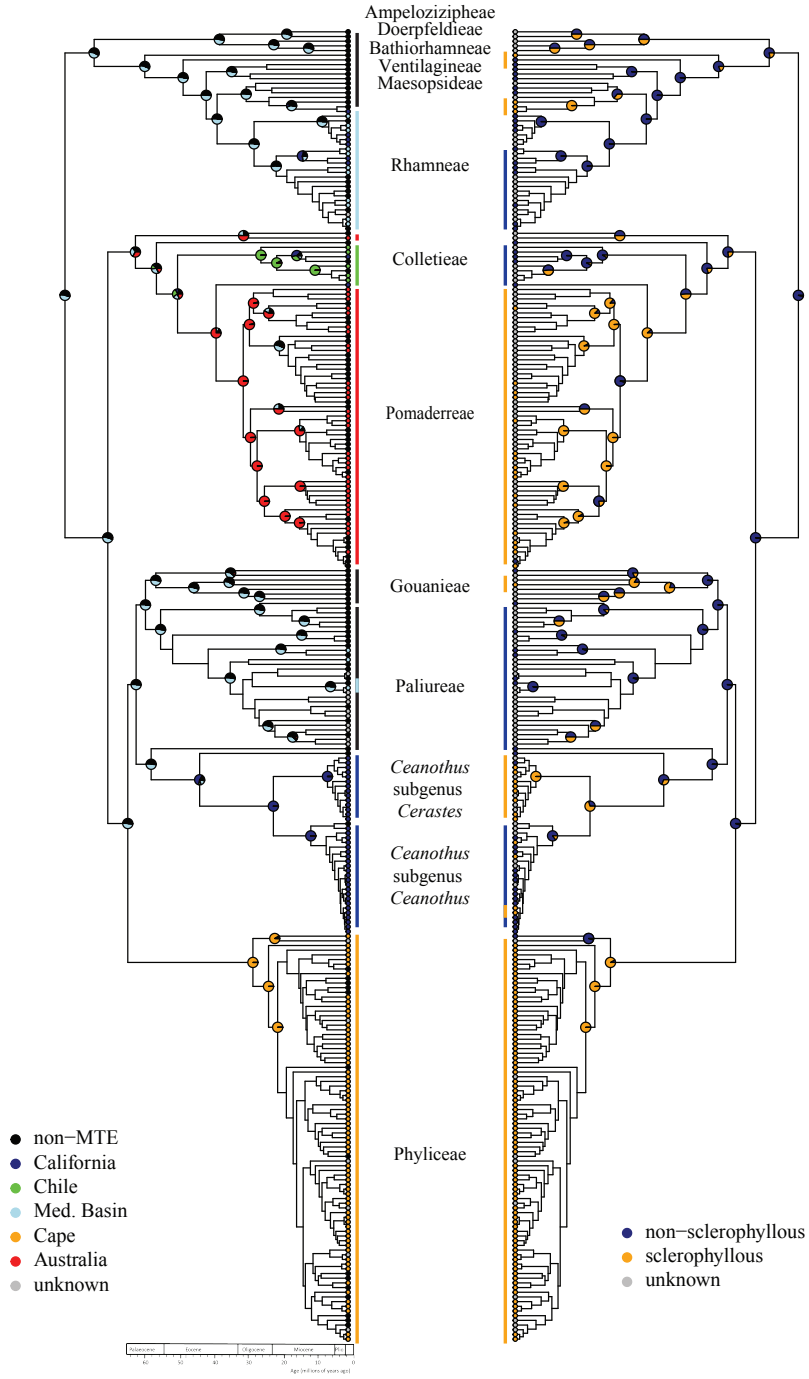

Figure 2
Ancestral state reconstructions as estimated by Bayesian MCMC over 100 trees, summarized on the Rhamnaceae MCC tree. Ancestrally, Rhamnaceae probably did not occur in MTEs ('black') (or respective areas), and the colonization of areas of the Cape, Australia, Chile, California and the Mediterranean Basin happened independently. The non-sclerophyllous character syndrome is ancestral in Rhamnaceae, and therefore probably pre-adaptive or exaptive to the areas of California, Chile and the Mediterranean Basin. The sclerophyllous character syndrome seems to have evolved contemporaneous with the colonization of the areas of the Cape and Australia, however, as colonization of these areas happened before the onset of the Mediterranean climate, this syndrome is likely exaptive to the Mediterranean climate.

Table 1

Transition rates between MTEs and character syndromes for the correlated (dependent) model of evolution resulting from maximum likelihood (ML) and Bayesian (MCMC) analyses in BayesTraits.

		q12	**q13**	**q21**	**q24**	**q31**	**q34**	**q42**	**q43**
	MTEs	0	0→1	0	0→1	1→0	1	1→0	1
	Traits	0→1	0	1→0	1	0	0→1	1	1→0
		Trait innova-tion outside MTE	MTE coloniza-tion absence trait	Losing trait outside MTE	MTE coloniza-tion presence trait*	Moving out of MTE absence trait	Trait innova-tion within MTE**	Moving out of MTE presence trait	Losing trait within MTE
CA + Sclerophyllous syndrome	ML	0.002	0.013	0.206	0.650	0.042	0.009	0.009	0.023
	MCMC	0	0.014	0.019	2.793	0.021	0.013	0.003	0.021
CCM + Non-sclerophyllous syndrome	ML	0.011	0.004	0.038	0.134	0.001	0.0002	0.336	0.019
	MCMC	0.008	0.003	0.031	0.024	0.004	0	0.031	0.03

Note. The numbers are averaged over 100 trees and the median for the six independent MCMC runs. *exaptation, **adaptation. CA=Cape/Australia, CCM=California/Chile/Mediterranean Basin.

Table 2

Bayes Factor (BF) test for adaptation and exaptation of sclerophyllous and non-sclerophyllous character syndromes in MTEs.

	Harmonic mean full model (q34 ≠ q12, q24 ≠ q13)	**Harmonic mean no adaptation (q34 = q12)**	**Harmonic mean no exaptation (q24 = q13)**	**Log (BF) adaptation *versus* no adaptation**	**Log (BF) exaptation *versus* no exaptation**	**Conclusion**
CA + Sclerophyllous syndrome	-139.923	-138.162	-139.813	-3.523	-0.221	No support for adaptation or exaptation
CCM + Non-sclerophyllous syndrome	-110.37	-111.364	-112.21	1.989	**3.681**	Support for exaptation

Note. Comparing the harmonic mean averaged over six independent runs of a model in which transition rates related to adaptation and exaptation (see table 1 and 4) are constrained, to the non-constrained (full) model. CA=Cape/Australia, CCM=California/Chile/Mediterranean Basin.

Selective trait optima in MTEs

For all traits - log (SLA), log (leaf area), NMDS component 1 and NMDS component 2 - a OU model with optima defined by CA, CCM and non-MTEs was strongly favoured over the other two models, indicated by the ΔAICc and the Akaike Weight, which reflects the relative likelihood of this model compared to the other models (table A5). The trait optima, 'θ', between CA and CCM/non-MTEs are clearly distinct for SLA, leaf area and NMDS component 1, which is also reflected by the small standard errors associated with the estimates of the mean (table 4). CA lineages are characterized by much lower SLA, much smaller leaves and a higher score on NMDS component 1, than CCM/non-MTE lineages. However, CCM MTEs and non-MTEs differ only slightly for these traits. The strength of attraction toward θ, 'α' (in 'per Myr'), and the rate of stochastic evolution away from the optimum, 'σ²' (for example for leaf area in 'mm²/Myr'), are the same for CCM, CA and non-MTEs as initially

stated in the models. These values indicate a relatively weak pull towards the trait optima - the phylogenetic half-life (ln(2) / α) is the time it takes to get halfway towards the optimum - and low rates of stochastic evolution, suggesting that trait changes are relatively slow over evolutionary time (table 4). For example, the Rhamnaceae crown age was estimated to be 84.1–100.6 Myr old (95% Highest Posterior Density, HPD) (Onstein et al. 2015) and it takes ~13.81 Myr to evolve halfway from relatively large microphyllous leaves (~524 mm²) to smaller leptophyllous leaves (~8.8 mm²). Although CCM show on average the highest score on NMDS component 2 (fig. 1), the optimum estimates in this model suggest that non-MTE lineages may actually have even higher scores. Nevertheless, the very high estimate of α suggests that the pull towards the optima is strong, and change could therefore happen rapidly (only 0.18 Myr to evolve halfway).

Table 3

Support for hypotheses concerning adaptation and exaptation of sclerophyllous and non-sclerophyllous character syndromes in MTEs.

Expectation	Interpretation	CA	CCM
q34 > q12	the rate of innovation of the character syndrome is higher within MTEs than outside MTEs (adaptation)	True n.s.	False n.s.
q24 > q13	the rate of transition into MTEs is higher when the character syndrome is present than when absent (exaptation)	True n.s.	**True**

Note. Adaptation and / or exaptation of the sclerophyllous and non-sclerophyllous character syndromes in Cape/Australia (CA) and Californian/Chilean/Mediterranean Basin (CCM) MTEs respectively. n.s. non-significant.

Table 4

Parameter estimates of the OU model with different selective optima in MTEs for traits associated with the sclerophyllous and non-sclerophyllous character syndromes.

Trait	α	Phylogenetic half-life ln(2)/ α	σ²	θ CA	θ CCM	θ non-MTE
Log (SLA)	0.0797	8.70	0.0088	0.5656 (se=0.05)	0.8992 (se=0.06)	0.9611 (se=0.04)
Log (Leaf area)	0.0502	13.81	0.0463	0.9446 (se=0.21)	2.0363 (se=0.23)	2.72 (se=0.13)
NMDS component 1	0.1147	6.04	0.0134	0.5065 (se=0.04)	-0.1935 (se=0.06)	-0.0802 (se=0.05)
NMDS component 2	3.8321	0.18	0.3041	-0.1077 (se=0.02)	-0.0833 (se=0.03)	-0.0023 (se=0.03)

Note. θ = selective optimum, α = the strength of selection toward θ, σ²= the rate of stochastic evolution away from θ, se=standard error, CA=Cape/Australian, CCM=California/Chile/Mediterranean Basin.

Effects of Mediterranean character syndromes and MTEs on diversification rates

The best fitting MuSSE model for the CA and the sclerophyllous character syndrome supported a model with positive additive effects of both speciation and extinction rates, and an interaction for extinction rates (table A2). This contrasts with the best fitted MuSSE model for CCM and the non-sclerophyllous character syndrome, which, like the CA model, supported a model with positive additive effects, but had an interaction for either speciation (model 1) or extinction rates (model 2) (table A1). Transition rates for both analyses were also included in the model, but some were set to 0 when this did not affect the model fit.

The parameters (table A5, fig. 3A) indicate an overall decrease in speciation rate for CA

lineages compared to non-sclerophyllous/non-CA lineages, and a higher speciation rate for sclerophyllous lineages compared to non-sclerophyllous/non-CA lineages. Extinction rates for CA lineages are much lower, and slightly higher for sclerophyllous lineages, than for non-sclerophyllous/non-CA lineages. Interestingly, the negative interactive effect (Table A5) suggests that a combination of occurring in CA and having the sclerophyllous syndrome decreases the extinction rate considerably, resulting in high net diversification rates. These results are consistent over topological and branch-length variation (fig. 3A).

Furthermore, our results (table A5, fig. 3B and 3C) indicate an overall increase in speciation and extinction rates for CCM lineages compared to sclerophyllous/ non-CCM lineages, but the non-sclerophyllous syndrome has a negative effect on the speciation rate, and no effect on the extinction rate, compared to sclerophyllous/ non-CCM lineages. Interestingly, model 1 suggests that lineages occurring in CCM and having the non-sclerophyllous syndrome have increased speciation rates compared to the opposite states, whereas model 2 indicates a high extinction rate for the interactive effect. Whether it is due to decreased speciation rates or increased extinction rates, overall diversification of lineages with non-sclerophyllous traits in CCM seems to be low compared to sclerophyllous lineages elsewhere, although these results are sensitive to topology and model (fig. 3B and 3C).

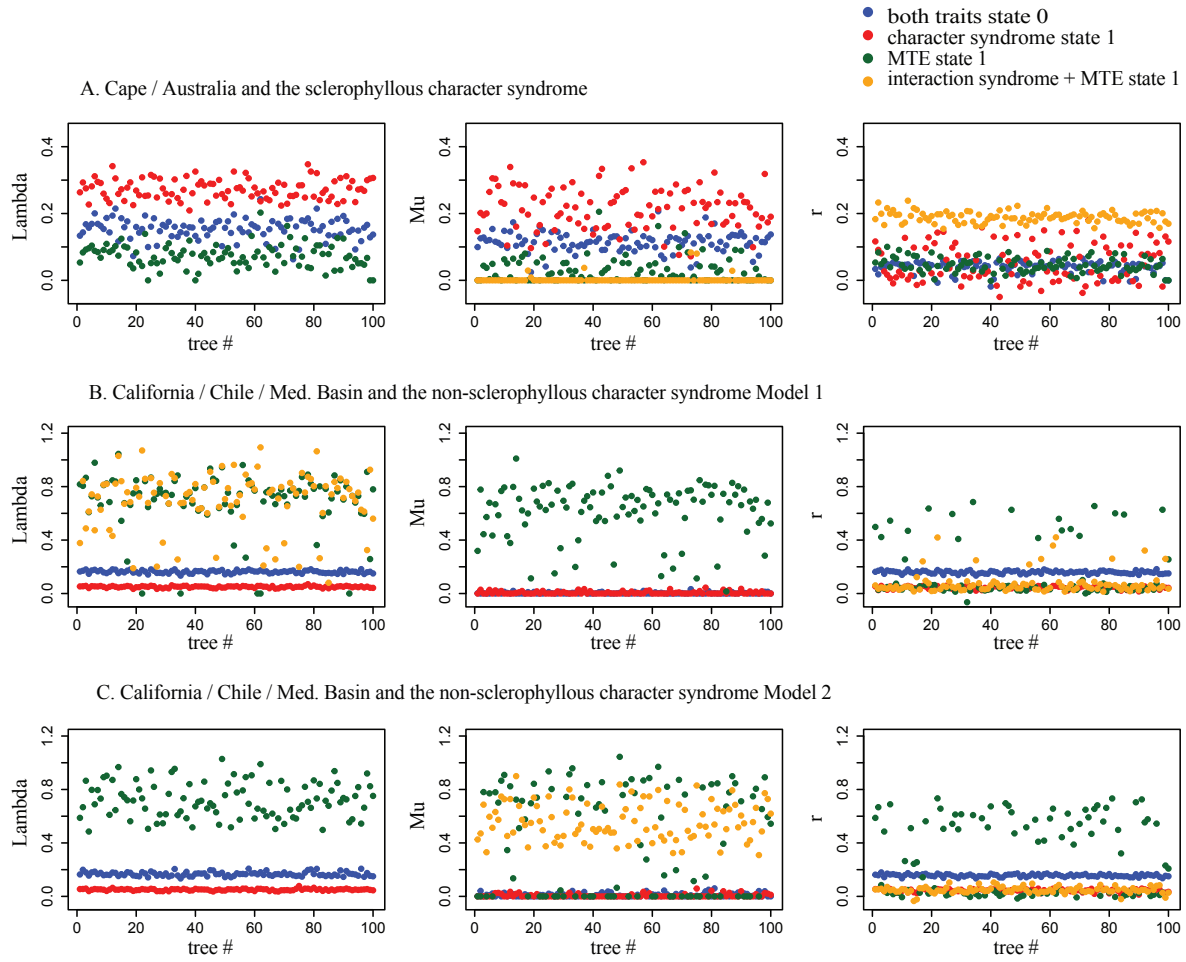

Figure 3

Estimates of lambda, mu and r (net diversification rate) for the Cape/Australian MTEs and the sclerophyllous character syndrome (A) and for the Californian/Chilean/Mediterranean Basin MTEs and the non-sclerophyllous character syndrome model 1 (B) and model 2 (C) over 100 trees. Net diversification rates are significantly higher in lineages with the interactive effects of the sclerophyllous character syndrome occurring in the shrublands of the Cape or Australia compared to any of these independently.

Discussion

Summary

We show that although there is a continuum in traits contributing to the sclerophyllous/non-sclerophyllous character syndromes, we can group Rhamnaceae species into either of these two categories (fig. 1). We show that sclerophyllous syndromes are largely found in the Cape and Australia, and in California it is represented by part of *Ceanothus*. The ancestral condition in Rhamnaceae was non-sclerophyllous, with sclerophylly evolving at least five times (fig. 2). The evolution of sclerophylly is strongly linked to Cape and Australian shrublands, and seems to have led to accelerated diversification through low extinction rates in these MTEs (fig. 3).

Character syndromes

The two clusters distinguishing the sclerophyllous and non-sclerophyllous character syndromes in the NMDS correspond to the original classification of Mediterranean character syndromes by Herrera (1992). These clusters distinguish species with sclerophylly in Cape and Australian MTEs from the non-sclerophylls in Californian, Chilean and Mediterranean Basin MTEs (fig. 1). The exception is the Californian *Ceanothus* subgenus *Cerastes* which has also evolved sclerophyllous leaves (fig. 2). Indeed, *Ceanothus* subgenus *Cerastes* was previously shown to have lower SLA and much longer leaf life span, and higher expression of the heat shock protein coding gene - which is associated with high temperature stress - than subgenus *Ceanothus* (Knight and Ackerly 2001, Ackerly 2004b). Our - arbitrary but quantitative - syndrome classification by means of NMDS accurately distinguished between these two syndromes in *Ceanothus* (fig. 1 and 2), suggesting that our classification of character syndromes reflects a 'true' shift in functional strategy.

The sclerophyllous traits found in Rhamnaceae in the Cape and Australia *versus* the predominantly non-sclerophyllous traits found in California, Chile and the Mediterranean Basin are typical of these floras. Verdu et al. (2003) classified 53, 69 and 92 genera typical for the floras of California, Chile and the Mediterranean Basin respectively, into predominantly sclerophyllous or non-sclerophyllous leaves. They showed that 54%, 71% and 66% of the genera of each of these three regions have non-sclerophyllous leaves, indicating that non-sclerophylly dominates in these regions. In comparison, Cowling and Witkowski (1994) showed that the floras of Australia and the Cape are dominated by sclerophylls. They compared sclerophyllous/non-sclerophyllous leaves in plant communities at five localities in the Cape and Australia, and showed that in the Cape 55% is sclerophyllous (48–78%, depending on soil type), 31% is non-sclerophyllous and 14% is succulent. The comparable figures for the Australian vegetation are 55% sclerophyllous (0–76%, depending on soil type), 34% non-sclerophyllous and 11% succulent. Thus, consistent with our results for Rhamnaceae, the floras of California, Chile and the Mediterranean Basin seem to be generally dominated by non-sclerophylls, and those of the Cape and Australia by sclerophylls.

Selective regimes

The sclerophyllous character syndrome seems to have evolved with the colonization of the Cape and Australia, as indicated by strong support for correlated evolution (table A5) and ancestral state reconstructions (fig. 2). However, we did not find significant statistical support for these traits to be evolutionary adaptations to Cape and Australian MTEs (table 2 and 3), as the shifts to the Cape and Australia co-occur with shifts to sclerophyllous syndromes on the phylogeny, and it is therefore unclear whether or not the traits evolved prior to the colonization of the Mediterranean environment. Nevertheless, trait values of SLA and leaf area seem to evolve towards certain optima in Cape and Australian shrublands, different from optima in these traits elsewhere (table 4), suggesting that at least some degree of selection may have taken place.

The selective regime that led to sclerophylly must have existed at the time sclerophylly evolved. Our ancestral state reconstructions suggest that the independent colonization of the Cape and Australia, and consequently the transition to sclerophylly, happened in both regions during the Oligocene: in the Cape 31.1 Ma (95% HPD: 22.7 – 41.7) and in Australia 34.3 Ma (95% HPD: 28.1 – 40.9). This 'old' age of sclerophyllous traits in the floras of Australia and the Cape is supported by both fossil and phylogenetic evidence (Jordan and Hill 1996, Hill 1998, Crisp and Cook 2013, Sniderman et al. 2013, Onstein et al. 2014). The summer drought regimes in Australia and South Africa evolved only during the Miocene (Hopper and Gioia 2004, Martin 2006, Cowling et al. 2009, Dupont et al. 2011), suggesting that scleromorphic traits pre-date the Mediterranean climate. Consequently we can reject sclerophylly as a response to summer-drought (fig. 2).

As noted by Crisp and Cook (2013), the selective regime leading to sclerophylly in the MTEs of the Cape and Australia may be infertile, low nutrient soils. The Cape and Australia are dominated by 'OCBILs' (old, climatically buffered, infertile landscapes) with very few 'YODFELs' (young, often disturbed, fertile landscapes) (Hopper 2009). This contrasts with the other three MTEs, which are dominated by YODFELS (Cowling et al. 2014). OCBILs probably provide the selective regime for the evolution of the sclerophyllous syndrome. It is difficult to obtain age estimates of the OCBILs. In the Cape these may date back to the Late Cretaceous, when rapid erosion of the escarpment had excavated the Cape fold mountains with their resistant sandstones (Tinker et al. 2008). The Cenozoic in the Cape was characterized by low denudation rates and geomorphic stability, resulting from the very erosion-resistant sandstone fold mountains of the Cape (Scharf et al. 2013). The situation in Australia is less clear. The Australian sandplains in their present expression, which host the typical kwongan vegetation, are hypothesized to date back not later than the Late Miocene (Wyrwoll et al. 2014). They reflect the long-term denudation history of the region, and the absence of Cenozoic glaciation (where ice-sheets could remove the pre-Quaternary regolith cover) and tectonic stability may have allowed these weathering products to be retained in the landscape. However, this relatively 'young' age does not preclude the existence of pre-Miocene environments analogous to present-day sandplains (Wyrwoll et al. 2014), and most parts of Australia have soils that are derived from substrates such as ancient sandstones and granites that are very deficient in major plant nutrients and often weather very slowly. Long periods of leaching in stable conditions may have led to extremely deficient soils and the same is likely to have been broadly true for large areas of the continent throughout the last 100 million years (Fox 1995). Consequently, the OCBIL habitats in the Cape and Australia most likely preceded the evolution of Rhamnaceae sclerophylls.

The deeply weathered infertile soils in the ancient, climatically buffered landscapes of the Cape and Australia, specifically the low availability of phosphorus (P) and nitrogen (N) and the relatively high N:P ratio (Lambers et al. 2010), may have selected for sclerophyllous traits in the Phyliceae and Pomaderreae. Sclerophyllous leaves consequently have very low P, low N, and a relatively high N:P ratio, limiting plant productivity and growth. A low SLA, or the inverse, a high leaf mass per area (LMA) is the direct result of this, and a typical feature of plants growing in nutrient-poor habitats (Lambers and Poorter 1992, Wright et al. 2002). Accumulation of fibre, thick cell walls and sclerenchyma, as well as quantitatively important secondary plant compounds, has been shown to increase lifespan and provide structural defence against herbivores and abiotic stress (Wright and Cannon 2001). These sclerophyllous traits in the Phyliceae and Pomaderreae may have provided an advantage when climate changed and aridification and fire activity expanded the heathlands in the Late Miocene/Pliocene (Keeley et al. 2012), making them 'exaptive' to aridity.

Sclerophylly in *Ceanothus* subgenus *Cerastes* may have a different underlying selective regime than infertile soils, as *Ceanothus* species with sclerophyllous and non-sclerophyllous syndromes can grow adjacent to each other (Davis 1999), and occupy similar environmental niches (Knight and Ackerly 2001). The differences in leaf morphology between subgenera *Ceanothus* and

Cerastes are associated with strategies of regeneration following fire. Species of subgenus *Cerastes* are generally obligate seeders, while species of subgenus *Ceanothus* are resprouters. Obligate seeders do not develop deep root systems and are more drought-tolerant, while resprouters develop deeper root systems over time and are more sensitive to water stress.

Hyperdiversity of sclerophylls in the Cape and Australia

The dominance of sclerophylls in the Cape and Australian floras might be due to sclerophyllous lineages showing higher diversification rates than non-sclerophyllous lineages (fig. 3, Byrne et al. 2011, Onstein et al. 2014). We concur with Crisp and Cook (2013) that progressive diversification and low extinction rates have led to present-day hyperdiversity of sclerophyllous taxa in these MTEs (Crisp et al. 2004, Hopper and Gioia 2004, Sniderman et al. 2013, Onstein et al. 2015). The alternative hypothesis, that 'explosive radiations' when Mediterranean climates were established in the Late Cenozoic (Cowling et al. 1996, Goldblatt and Manning 2002) would have led to the increase of sclerophyllous taxa due to rapid speciation (McLoughlin and Hill 1996), can be rejected for Rhamnaceae.

This match of trait and environment could facilitate survival, reducing extinction rates over time. The high diversification rate of sclerophyllous Rhamnaceae in the Cape and Australia indeed seems to be primarily driven by a reduction in extinction rate of these lineages compared to non-sclerophylls and non-CA lineages (fig. 3). Although we argue that sclerophylly was selected for on old, oligotrophic soils, these 'hardy' leaves may have provided an additional advantage when Mediterranean type climates with dry summers became established in the Miocene–Pliocene, thereby fostering low extinction over time. Such a match of traits and environment leading to rapid diversification was previously shown for the Cape Phyliceae, Penaeaceae and Diosmeae (Onstein et al. 2014). However, in this study (Onstein et al. 2014) the occurrence of low SLA, small leaves, and occurring in fynbos could not be disentangled, and it was therefore unclear whether the habitat (fynbos), the traits (low SLA, small leaves) or a combination of both led to higher diversification rates compared to their high SLA, large leaved Afromontane forest sister-groups. However, this phenomenon is not restricted to MTEs: Bouchenak-Khelladi et al. (2014) investigated diversification rates of grasses with CO_2-concentrating mechanisms (versus C3 photosynthesis) inhabiting dry and open environments (versus wet and shady) and found a significant, positive, interaction effect of these two characters on diversification rates. Similarly, low SLA leaves increased diversification rates of Ericaceae in nutrient-poor mountain habitats (Schwery et al. 2014). Evidently, both intrinsic and extrinsic variables are important when it comes to understanding the triggers of evolutionary radiations (Bouchenak-Khelladi et al. 2015).

The dominance of sclerophyllous leaves in the Cape and Australia might well be a macro-evolutionary consequence of the increased diversification rate of lineages with these traits. If sclerophyllous leaves only give an ecological advantage, then there is no reason why most species are sclerophyllous – it could simply result in one very common, widespread, ecologically dominant sclerophyllous species, and with the diversity being non-sclerophyllous. Here we propose an evolutionary mechanism leading to the floristic dominance of a vegetative trait.

Conclusion

Our results suggest that common climate is not enough to explain convergence and non-convergence of vegetation physiognomy in Mediterranean-type ecosystems (Cowling and Witkowski 1994) and we suggest that soil nutrient status may be -partly- responsible for this. Soil nutrient status can influence vegetation type, plant physiognomy and plant community boundaries, as was shown for the Brazilian cerrado (Goodland and Pollard 1973) and the Cape fynbos (Richards et al. 1997). The shift to

sclerophylly in the Cape and Australia was linked to increased diversification, which, if this is a general feature of sclerophyllous lineages in these MTEs, may have consequently generated a flora dominated by sclerophyllous species, even if the deeper phylogenetic diversity might be non-sclerophyllous.

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Supporting Information Chapter IV

Appendix A

Table A1. MuSSE multistate model testing the joint effect of California / Chile / Mediterranean Basin and the non-sclerophyllous character syndrome on speciation and extinction rates.

Model	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
Simple	6	-1156.9	2325.8		
Additive λ	8	-1156.9	2325.8	82.346	< 2.2e-16 ***
Additive μ	8	-1130.0	2276.1	53.701	2.183e-12 ***
Additive q	10	-1148.7	2317.4	16.433	0.00249 **
Additive λ and μ	10	-1100.3	2220.7	113.13	< 2.2e-16 ***
Additive λ, μ and q	14	-1088.8	2205.7	23.014	0.0001258 ***
Additive λ, μ and q + interaction λ	**15**	**-1085.8**	**2201.6**	**6.1005**	**0.01351 ***
Additive λ, μ and q + interaction μ	**15**	**-1085.9**	**2201.9**	**5.7915**	**0.0161 ***
Full (Additive λ, μ and q + interaction λ and μ)	16	-1087.8	2207.6	-4.0619	1
Multistep Additive λ, μ and q + interaction λ	19	-1085.8	2209.5	0.34828	0.9865
Multistep Additive λ, μ and q + interaction μ	19	-1088.5	2214.9	-5.3452	1
Multistep full model	20	-1087.9	2215.7	-0.095607	1

Note. Testing the joint effect of character syndromes and MTEs on speciation and extinction rates. Models (nested) were compared with a likelihood-ratio test; de model with the least number of parameters without a significant decrease in log likelihood (LnLik) was selected (in bold). Df=degrees of freedom, AIC= Akaike information criterion, ChiSq= Chi-square, Pr(>|Chi|) indicates whether this model is significantly different from the more simplistic model. Signif. codes: 0 '***' 0.001 '**' 0.01 '*', λ=speciation rate; μ=extinction rate, q=transition rate. NA= not applicable. "Multistep" included parameters for transition rates that imply simultaneous changes in more than one trait.

Table A2. MuSSE multistate model testing the joint effect of Cape / Australia and the sclerophyllous character syndrome on speciation and extinction rates.

Model	Df	lnLik	AIC	ChiSq	Pr(>|Chi|)
Simple	6	-1186.6	2385.2		
Additive λ	8	-1168.0	2352.0	37.238	8.199e-09 ***
Additive μ	8	-1162.5	2340.9	48.284	3.276e-11 ***
Additive q	10	-1179.1	2378.3	14.935	0.004837 **
Additive λ and μ	10	-1163.6	2347.2	45.956	2.515e-09 ***
Additive λ, μ and q	14	-1150.8	2329.6	25.639	3.741e-05 ***
Additive λ, μ and q + interaction λ	15	-1149.0	2328.0	3.5595	0.05921
Additive λ, μ and q + interaction μ	**15**	**-1147.3**	**2324.5**	**7.0923**	**0.007742 **
Full (Additive λ, μ and q + interaction λ and μ)	16	-1152.1	2336.2	-6.1485	1
Multistep Additive λ, μ and q + interaction λ	19	NA	NA	NA	NA
Multistep Additive λ, μ and q + interaction μ	19	-1150.9	2339.7	-7.2028	1
Multistep full model	20	-1148.6	2337.2	6.9964	0.1361

Note. Models (nested) were compared with a likelihood-ratio test; de model with the least number of parameters without a significant decrease in log likelihood (LnLik) was selected (in bold). Df=degrees of freedom, AIC= Akaike information criterion, ChiSq= Chi-square, Pr(>|Chi|) indicates whether this model is significantly different from the more simplistic model. Signif. codes: 0 '***' 0.001 '**' 0.01 '*', λ=speciation rate; μ=extinction rate, q=transition rate. NA= not applicable. "Multistep" included parameters for transition rates that imply simultaneous changes in more than one trait.

Table A3. Bayes Factor (BF) test for correlated evolution between MTEs and corresponding character syndromes in BayesTraits.

	Harmonic mean dependent model	**Harmonic mean independent model**	**Log (BF)**	**Conclusion**
Cape/Australia + Sclerophyllous syndrome	-139.923	-149.282	**18.717**	Very strong support for correlated evolution
California/Chile/Med.Basin + Non-sclerophyllous syndrome	-110.37	-116.551	**12.362**	Very strong support for correlated evolution

Note. Comparing the harmonic mean averaged over six independent runs of a dependent (correlated) model to an independent (uncorrelated) model.

Table A4. Comparison of models of evolution (BM, OU) for traits associated with the sclerophyllous and non-sclerophyllous character syndromes.

Trait	**ΔAICc**			**Akaike Weight**		
	BM	**OU 1-optimum**	**OU 3-optima**	**BM**	**OU 1-optimum**	**OU 3-optima**
Log (SLA)	71.82	18.54	0	2.53 e-16	9.39 e-05	~1
Log (Leaf area)	42.29	19.93	0	6.56 e-10	4.71 e-05	~1
NMDS component 1	87.94	43.41	0	7.98 e-20	3.75 e-10	~1.0
NMDS component 2	153.44	2.95	0	3.9e-34	0.186	0.814

Note. AIC= Akaike Information Criterion, Akaike Weight represent the relative likelihood of a model, BM = Brownian Motion, OU = Ornstein-Uhlenbeck.

Table A5. Maximum Likelihood estimates of speciation (λ), extinction (μ) and transition (q) rates between Cape/Australian MTEs (M) and the sclerophyllous character syndrome (S) (CA) and Californian/Chilean/Mediterranean Basin MTEs (M) and the non-sclerophyllous character syndrome (S) (CCM m1 and m2) based on the preferred models.

	λ0	**λM**	**λS**	**λMS**	**μ0**	**μM**	**μS**	**μMS**	**qM0→ 1.0**	**qM0→ 1.S**
CA	0.134	-0.080	0.130		0.100	-0.100	0.047	-0.047	0	0
CCM m1	0.165	0.653	-0.111	-0.328	0.002	0.317	-0.002	NA	0.001	0.010
CCM m2	0.164	0.423	-0.110	NA	0.002	-0.002	-0.002	0.427	0.001	0.012

	qM1→ 0.0	**qM1→ 0.S**	**qS0→ 1.0**	**qS0→ 1.M**	**qS1→ 0.0**	**qS1→ 0.M**
CA	0.040	-0.003	0.008	-0.004	0.097	-0.097
CCM m1	0	0.023	0.002	0.237	0.002	0.002
CCM m2	0	0.020	0.002	0.279	0.002	0

Note. MS refers to the interaction term between the area and the character syndrome. State '0' refers to sclerophyllous/non- Californian/Chilean/Mediterranean Basin lineages. '→' indicates the transition to another state. NA= not applicable.

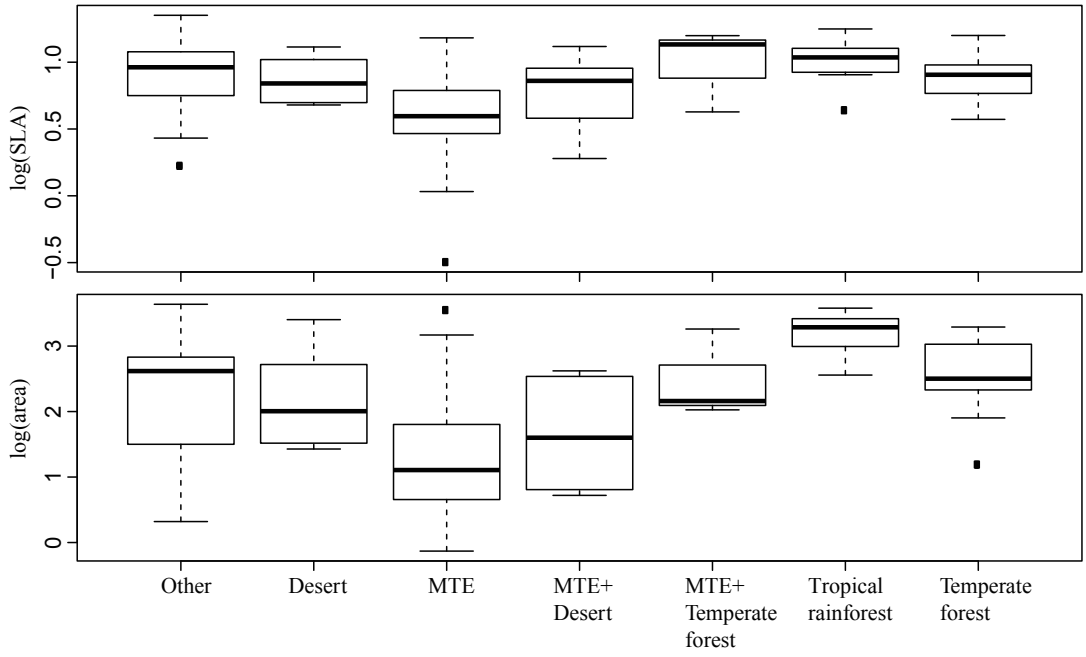

Figure A1. Box-and-whisker plot indicating the median, the lower and upper quartiles (25% and 75%), the minimum and maximum values (excluding outliers), and outliers (any value that lies more than one and a half times the length of the box from either end of the box) for log (SLA) and log (leaf area) for each biome for Rhamnaceae. The ANOVA indicates a significant reduction of log (SLA) in MTEs ($y = 0.9 – 0.29x$). For log (leaf area), the ANOVA indicates a significant reduction in MTEs ($y= 2.22 – 0.92x$) and a significant increase in tropical rainforests ($y= 2.22 + 0.97x$). When considering the standard error, we make the binary cut-off for log (SLA) in MTEs at <0.69 and for log (leaf area) at <1.66.

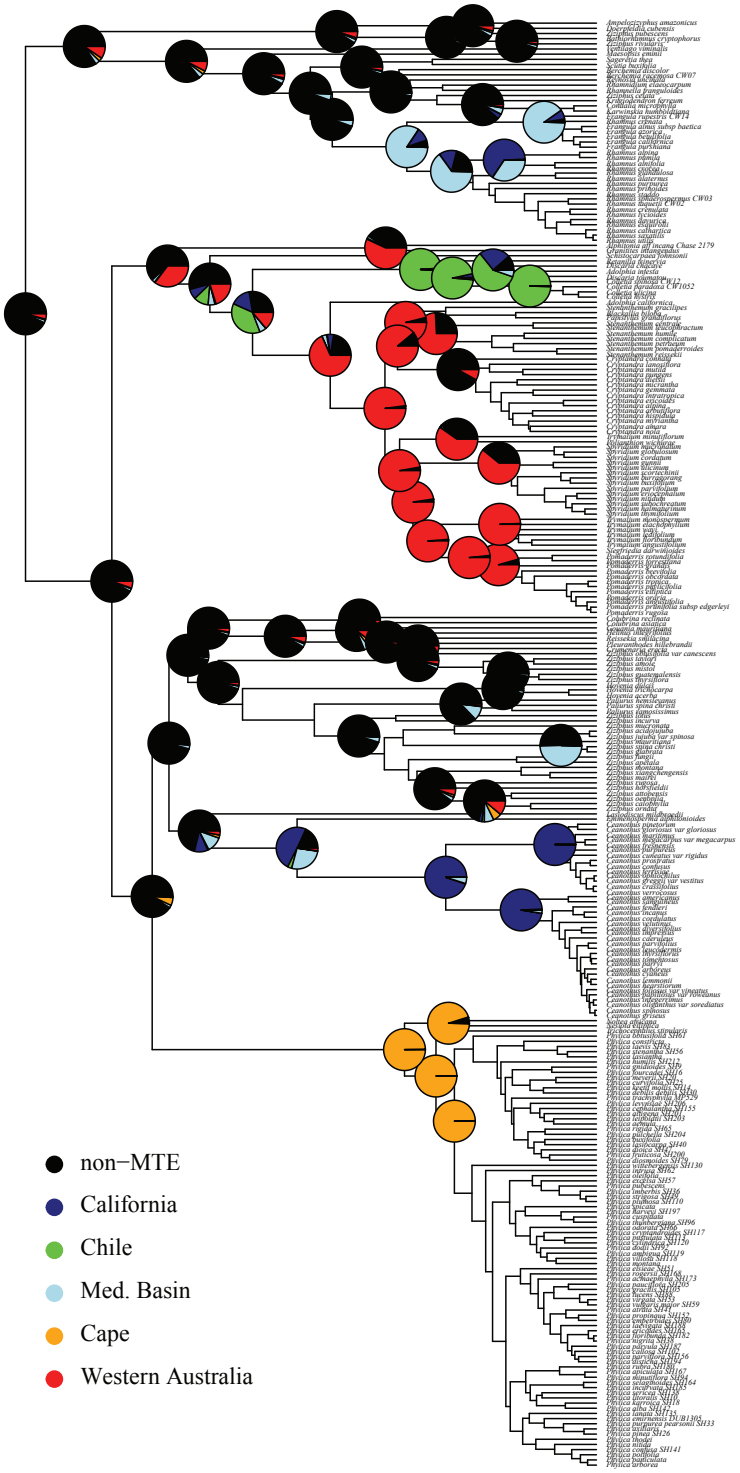

Figure A2. Ancestral state reconstruction of MTE area as estimated by Maximum Likelihood over 100 trees, summarized on the Rhamnaceae MCC tree. Ancestrally, Rhamnaceae probably did not occur in MTEs ('black') (or respective areas), and the colonization of areas of the Cape, Australia, Chile, California and the Mediterranean Basin happened independently.

CHAPTER V: NICHE AND TRAIT EVOLUTIONARY RATES ARE CORRELATED DURING THE PROTEACEAE RADIATION

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Author contributions:

REO, GJ, HS and HPL designed the research; REO collected functional trait data; IA assisted during trait measurements; REO, GJ and PW performed fieldwork; GJ collected distribution and climate data; YBK assembled the sequence alignment and performed preliminary phylogenetic analyses; RC checked fossil calibrations; REO, HS and PW performed phylogenetic dating analyses; REO performed principal component analyses, geometric morphometrics, phylogenetic generalised least squares regression, evolutionary model testing in OUwie and morphological rate analyses in BAMM; REO wrote the manuscript, with major comments from HPL, GJ, HS and RC, and minor comments from YBK.

Abstract

Ecologically-driven diversification can create spectacular diversity in both species numbers and form. However, the prediction that rates of change in intrinsic (e.g. functional trait) and extrinsic (e.g. climatic niche) variables are coupled during evolutionary radiation has not been critically tested, even though it is a central prediction of the model. Here, we test this hypothesis in the Southern Hemisphere angiosperm family Proteaceae, which occupies habitats ranging from tropical rainforests to deserts, shows spectacular radiations in open, Mediterranean shrublands in the Cape Floristic Region (CFR) and the Southwest Australian Floristic Region (SWAFR), and is remarkably variable in leaf morphology. We built a phylogeny for 337 Proteaceae species (21% of total), representing all main clades, climatic tolerances and morphologies, and collected leaf functional trait data (blade area, sclerophylly, leaf shape) for 261 species and climatic niche data for 1645 species. We used phylogenetic generalized least squares regression, quantitative-trait evolutionary model testing and estimates of rates of functional trait and climatic niche evolution to show that divergent selection may have caused lineages in open vegetation types to evolve towards trait and climatic niche optima distinct from those from closed forest. Furthermore, we show that the macro-evolutionary rates of functional trait and climatic niche evolution are strongly correlated, and that these rates are particularly high in open vegetation clades, such as *Banksia* and *Grevillea* in the SWAFR, and the Proteeae and Leucadendreae in the CFR, compared to rates in closed forest clades such as *Macadamia* and Roupalinae. We argue that exposure to variable climatic micro-environments in Mediterranean shrublands favours higher interspecific trait variability, and this may have facilitated the radiations in these systems.

Keywords:

Functional trait, Mediterranean-type ecosystem, evolutionary radiation, climatic niche

Introduction

The evolution of the enormous plant species richness of the southern Mediterranean type ecosystems – the Cape Floristic Region (CFR) of South Africa and the Southwest Australian Floristic Region (SWAFR) of Australia (Cowling et al. 1996) – remains enigmatic. It has, however, been demonstrated for several angiosperm clades that net diversification rates (speciation rate – extinction rate) are higher within the Mediterranean systems than in the adjoining subtropical or temperate biomes (Sauquet et al. 2009, Onstein et al. 2015, Reyes et al. 2015). This higher diversification rate may have resulted from low extinction rates (Sniderman et al. 2013, Onstein et al. 2015) and / or high speciation rates (Reyes et al. 2015). However, there is no consensus concerning the processes which have generated this diversity and could have 'triggered' and 'modulated' the radiations (*sensu* Bouchenak-Khelladi et al. 2015). Several hypotheses exist (Linder 2003, Hopper and Gioia 2004, Hopper 2009), related to the heterogeneity of the environments which could have partitioned niches and influenced diversification rates, such as pollinator (Johnson 1996), soil type (Schnitzler et al. 2011) and climatic niches (Carlson et al. 2011, Schnitzler et al. 2012). With respect to climatic niches, the absence of climatic smoothing caused by dense forests may result in greater spatial climatic heterogeneity in open (Mediterranean) vegetation. This 'modulation' by forests can dampen the variation in both temperature and rainfall compared to unforested areas, as well as ameliorate the effects of drought by recycling water, thereby reducing local fluctuations in climate (Chen et al. 1999, Clinton 2003). The absence of such modulation may create many more niches in which species can survive competition

and maintain a much higher diversity (Pyšek et al. 2002). Open type habitats may be much more sensitive to small spatial and temporal climatic variations, which are otherwise buffered by dense forest covers ('biotic modulation', Linder et al. 2012). This may consequently influence the range of climate-driven (leaf) adaptations found in open compared to forested vegetation. Therefore, we hypothesize that rates of intrinsic (e.g. functional trait) and extrinsic (e.g. climatic niche) change are coupled as lineages adapt to the unmitigated local climate, and we predict particularly fast rates in open compared to closed vegetation systems.

Here, we use the Southern Hemisphere angiosperm family Proteaceae Juss. to test this hypothesis. Proteaceae are excellent to test this hypothesis, as they show evolutionary radiations in open Mediterranean habitats (Sauquet et al. 2009) but also contain many lineages in closed rainforest habitats. Proteaceae comprise ~1700 species (Weston 2007), most of which (>1100 species) occur in open shrublands and sclerophyllous woodlands in Mediterranean climate zones (Weston 2007), especially in the CFR and SWAFR. This was shown to have probably resulted from higher net diversification rates of Mediterranean lineages, compared to non-Mediterranean lineages (Sauquet et al. 2009, Reyes et al. 2015, but see Valente et al. 2010 and Cardillo and Pratt 2013). Nevertheless, many Proteaceae clades are characteristic of rainforest habitats, mostly in tropical Australia (Queensland), but also in New Caledonia, South America and southern and eastern Asia. Other clades are widespread, for example the genus *Grevillea*, which occurs from alpine to desert to Mediterranean to tropical rainforest, in Australia, New Caledonia and New Guinea. Evidently, there have been many transitions between wet and dry climates (Jordan et al. 2008) and consequently there is a spectacular morphological variation within the family, from large forest trees with irregularly shaped, often lobed leaves as juveniles, much smaller simple leaves as adults, to small woody shrubs with needle-like or sharply toothed leaves (Weston 2007). This morphological diversity is also expressed in leaf and vein anatomy and scleromorphic structures (Jordan et al. 2005, Jordan et al. 2013).

To evaluate the roles of traits and climatic niches in the evolution of Proteaceae, we formulate four hypotheses. First, we hypothesize that climate is a strong predictor of species functional traits (Reich et al. 2003), and, second, we expect open (predominantly Mediterranean) and closed (predominantly tropical rainforest) vegetation types to cause divergence in trait optima. Third, we hypothesize that the macro-evolutionary rate of functional trait change (disparification) and climatic niche evolution (climatic tolerance) in Proteaceae are correlated, which, if true, would suggest that clades which exhibit faster niche evolution also diversify faster morphologically. Finally, we hypothesize that these correlated functional trait and climatic niche rates are higher in open vegetation clades than in to closed vegetation clades. This may be due to the greater spatial climatic variation in open vegetation. We postulate that the strong niche differentiation in open compared to closed habitats may have led to faster divergence rates, resulting in greater species richness in these systems than in closed forests. In this paper, we test these four hypotheses using a new species-level dated phylogeny of Proteaceae and a new compilation of functional and climatic niche data.

Materials and Methods

Functional traits and climate

We selected fourteen one-dimensional, quantitative leaf functional traits related to blade area, sclerophylly and leaf shape, which represent the main variation in leaf morphology in the family, and have been shown to be related to different ecological strategies (Table 1). These were measured for 261 Proteaceae species in 66 genera (data will be available from the Dryad Digital Repository). Measurements followed the protocols of Cornelissen et al. (2003) and Royer et al. (2005) and were performed on ten randomly chosen fully-expanded sunlit adult leaves from different individuals (if available). These leaves were collected from the wild in Jurien Bay (Western Australia), Robson

Creek (Queensland) (Bradford et al. 2014) and the Blue Mountains (New South Wales), and from botanic gardens in Kings Park Botanic Garden, Atherton Botanic Garden, the Royal Botanic Garden Melbourne, the Royal Botanic Gardens (Sydney, Mount Annan and Mount Tomah), and Kirstenbosch National Botanical Garden (South Africa). Leaves from the remaining 86 species were collected from herbarium specimens from the herbarium of the University of Zurich (Z). We performed an experiment in which we dried collections of fourteen Proteaceae species representing a wide range of leaf types, to evaluate the error associated with trait measurements using pressed and dried leaves. This measurement error was between 0% (dissection, perimeter) and 20% (circularity, effective leaf size) (results not shown). We assumed this error to be uniform in Proteaceae and used it to apply a correction to all measurements of non-fresh leaves. We calculated the mean trait value for each species after log-transforming all traits except from leaf circularity, to minimize the effect of outliers and obtain normality. In addition to these fourteen traits, we used geometric morphometric techniques with four landmarks representing variation in leaf length/width ratio and in obovate/elliptical leaf shapes analysed in MorphoJ (Klingenberg 2011). We performed a principal component analysis (PCA) on these landmarks and extracted species scores from principal component (PC) 1 and PC2 ('shape PC1' and 'shape PC2' hereafter). These PC scores were used in subsequent analyses.

To estimate the realized climatic tolerance for a given species, we retrieved GPS coordinates from the Global Biodiversity Information Facility (GBIF; http://gbif.org, accessed 6/8/2013) and the Protea Atlas (Rebelo 2006), supplemented by personal observations. All records were assessed for consistency with documented distributions of the species (for details see Jordan et al. 2013). This resulted in 38,892 unique data points representing 1643 species (i.e., 97% of all known species in the family). We then used WorldClim (Hijmans et al. 2005) and ANUCLIM (Xu and Hutchinson 2011) to extract the species median climate data for each of the 18 BIOCLIM variables, which describe the major temperature and precipitation dimensions of a given species. We selected eight climatic variables which we presumed to be important for plant growth and performance: mean annual temperature (MAT), maximum temperature warmest month (MaxTWarmMonth), minimum temperature coldest month (MinTColdMonth), mean precipitation in the coldest, the warmest, the wettest and the driest quarters of the year (PrecipColdestQ, PrecipWarmestQ, PrecipWettestQ, PrecipDriestQ) and mean annual precipitation (MAP). The square root of the precipitation variables was taken to approach normality in the data.

We used principal component analysis (PCA) to reduce the dimensionality in the climate and leaf morphology data, because individual variables may be correlated but not perfectly so. The two principal components which explained the highest percentage of the variation in the datasets were used in subsequent analyses ('climate PC1', 'climate PC2' and 'leaves PC1', 'leaves PC2' hereafter), in addition to the one-dimensional trait and climate data.

Phylogeny

In order to interpret the evolution of leaf form, we generated a new time-calibrated phylogenetic tree for Proteaceae based on sequences for 343 taxa, representing 339 Proteaceae species (337 species plus two subspecies), representing 75 (91%) out of the 82 recognized genera, 4 outgroup species from related families (Nelumbonaceae and Platanaceae) and more distant outgroups (Buxaceae, Sabiaceae) for five chloroplast markers (*atpB*, *matK*, *rbcL*, *rpl16* intron and *trnL-trnF*) downloaded from GenBank (GenBank accession numbers are provided in Table S1). Sequences were aligned using MUSCLE v3.6 (Edgar 2004). Alignments were checked by eye and ambiguous fragments were excluded from the analysis. The combined matrix included 6251 characters. Our main purpose here was not to estimate again the backbone tree of Proteaceae, but instead to produce a species-level phylogeny with as many species sampled as possible. Initial analyses revealed that molecular data (species representivity across multiple markers) were too fragmentary for an unconstrained analysis,

therefore we added monophyly constraints based on previous published studies in order to infer a meaningful phylogeny while maximizing species sampling. We constrained clades to be monophyletic if these had a support of posterior probabilities >0.95 and/or a bootstrap of >80 in the Proteaceae genus-level tree by Sauquet et al. (2009), the *Protea* tree by Valente et al. (2010a), the *Banksia* tree by Cardillo and Pratt (2013), the *Grevillea, Hakea* and *Finschia* trees by Holmes et al. (2014), Mast et al. (2012) and based on taxonomy by Barker et al. (1999), Olde and Marriott (1994) and Makinson (2000). In addition, all genera except *Grevillea, Mimetes, Persoonia* and *Stenocarpus* were constrained to be monophyletic based on morphology and ongoing, unpublished phylogenetic work (the constrained topology is provided in Fig. S1). We simultaneously estimated the topology and divergence times using BEAST v1.8 (Drummond et al. 2012a) performed on the CIPRES Science Gateway (Miller et al. 2010) under an uncorrelated lognormal relaxed-clock model, using the general time-reversible (GTR) substitution rate model and Γ-distributed rates among sites with a proportion of invariant sites to describe the rate heterogeneity among sites. We used fourteen critically selected fossils for calibration (Table S2). In addition to all of the phylogenetically analysed fossil pollen calibrations of Sauquet et al. (2009), we also used fossils suggested by Dettmann and Clifford (2005), Barker et al. (2007) and the review of leaf fossils by Carpenter (2012) (for more details on fossil selection and placement see Table S2). For all fossil minimum age constraints we used uniform prior distributions because we do not have a sufficiently densely sampled fossil record to estimate the fossil preservation probability and fossil constraints were placed conservatively on the lowest safe node of the clade where they were hypothesized to belong (Sauquet et al. 2012). In addition, the first occurrence of tricolpate pollen was used as a maximum age for the root node (125 Ma), on the assumption that eudicots are unlikely to have originated much earlier (reviewed by Friis et al. 2006). We ran seven independent Markov Chain Monte Carlo (MCMC) runs of 50×10^6 generations and checked for convergence between runs, and effective sample sizes of >200 for all parameters in Tracer 1.5 (Rambaut and Drummond 2007). We combined the seven runs in LogCombiner after discarding the first 10% generations (burnin) in each run, and we selected and annotated the Maximum Clade Credibility (MCC) tree using TreeAnnotator. The MCC tree was used in all subsequent comparative analyses after pruning the outgroup species and subspecies.

The effect of climate on trait variation

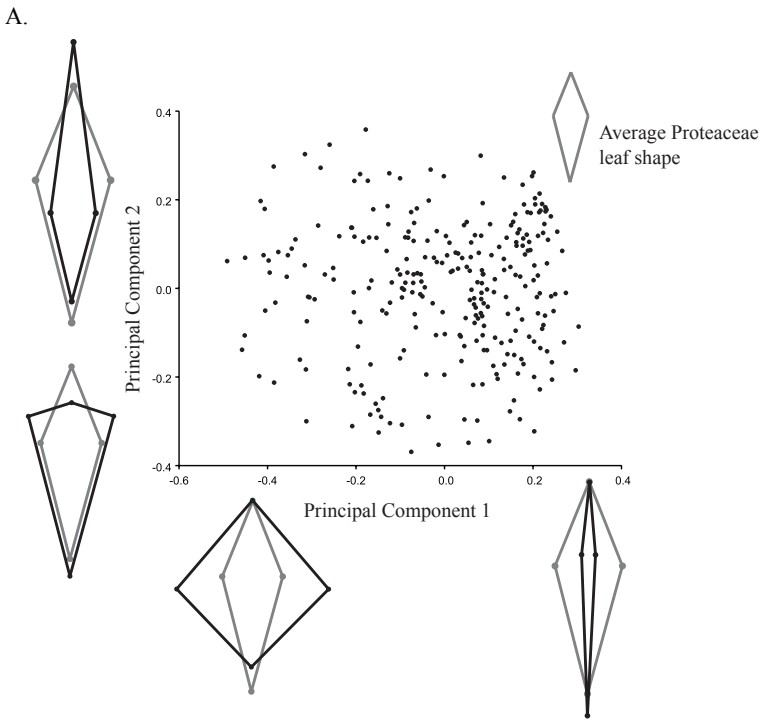
To assess whether variation in species mean functional traits is explained by variation in climate, we tested the effect of climate PC1 and PC2 (explanatory variables) on mean functional traits (response variable), for each trait separately. To account for any possible phylogenetic non-independence in the correlations among these variables, we fitted phylogenetic generalised least squares (PGLS) regression models using the 'pgls' function in the 'caper' library (Orme et al. 2013) implemented in R (R Development Core Team 2008). The PGLS method estimates phylogenetic signal and regression parameters simultaneously, adjusted for the phylogenetic signal in the model residuals: when absent $\lambda = 0$; when strong $\lambda = 1$. We chose the best transformation structure of the covariance matrix based on model fit, changing either lambda (λ) (i.e. the internal branch lengths of the phylogeny are multiplied by a constant), delta (δ) (i.e. all the values in the covariance matrix are raised to the power δ) or kappa (κ) (i.e. all branch-lengths in the phylogeny are raised to the power κ) and fixing the other parameters to 1 (Orme et al. 2013), provided that the best model met the model requirements: normality of residuals, and absence of relationships between the fitted values and both the residuals and the observed values.

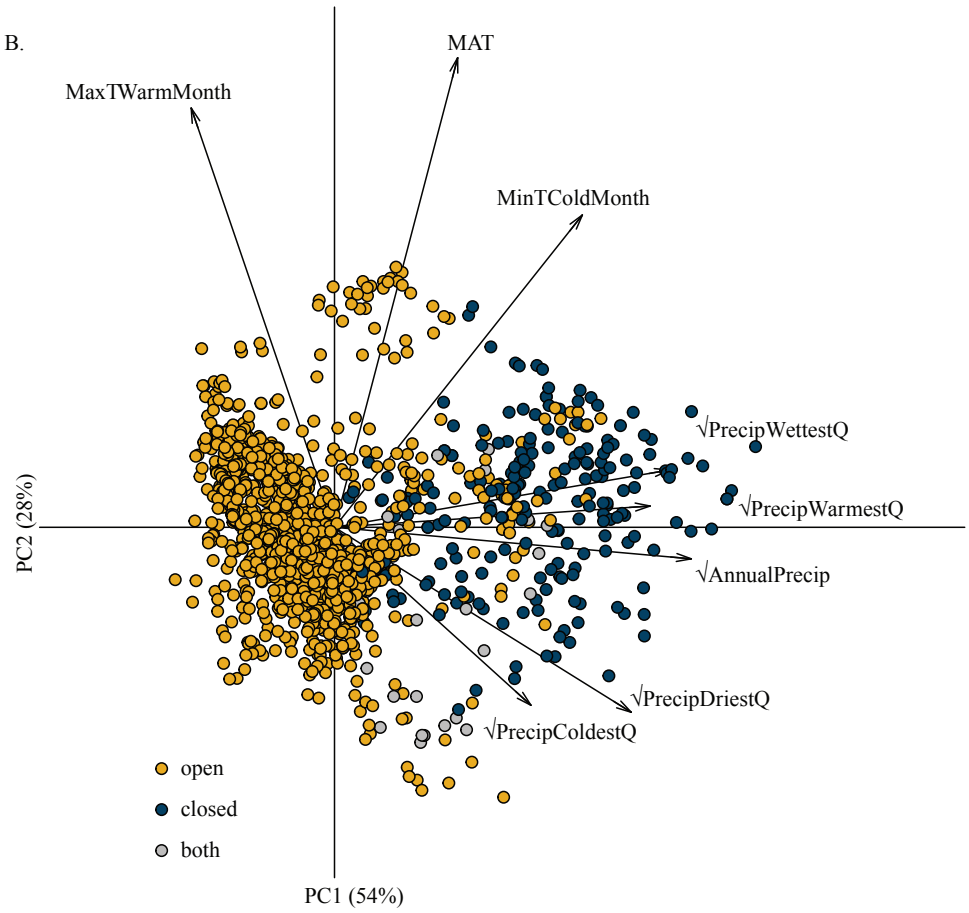
Table 1

Selection of leaf functional traits for Proteaceae and their hypothesized climate-related functionality based on the literature. Principal component (PC) 1 and PC2 resulting from the shape, leaf morphology and climate PCAs, and their functionality based on the association to one-dimensional climate and leaf traits, are indicated as well (last rows).

Trait	Definition	Functionality	Reference
Blade area	Area of leaf blade (mm^2) (excluding petiole)	Leaf size declines with increasing altitude, decreasing rainfall and soil nutrient content (i.e. dry and cold environments), as decreases in area come with smaller boundary layers and better convective heat exchange with the environment.	(McDonald et al. 2003)
Specific Leaf Area (SLA)	Leaf weight / leaf area (including petiole) (mg / mm^2)	Illustrating the 'leaf economic spectrum': low SLA 'sclerophyllous' leaves have long leaf lifespans, low photosynthetic rates, and low nitrogen contents.	(Wright et al. 2004)
Perimeter	Leaf perimeter (mm)	Measure of leaf interaction with the environment, i.e. leaf boundary. Correlated to blade area.	
Standardized Perimeter: Perimeter / Area	Perimeter / Area (mm^{-1})	Measure of leaf interaction with the environment, i.e. leaf boundary, corrected for leaf area.	
Complexity	Perimeter/[√(leaf area)] (dimensionless)	Shape describer (leaf dissection index).	(McLellan and Endler 1998)
Feret-diameter (effective leaf area)	Diameter of largest circle within blade area (mm)	Direct measurement of leaf boundary layer, related to convective heat exchange.	(Parkhurst and Loucks 1972)
Circle (effective leaf area)	Area of largest circle within blade area (mm^2)	Direct measurement of leaf boundary layer, related to convective heat exchange.	
Feret-diameter ratio	Major leaf axis length / Feret-diameter (dimensionless): $2*\sqrt{(area/\pi)}$/ Feret-diameter	Length:width ratio is positively correlated with temperature (Wolfe, 1993), Feret-diameter ratio may be affected both positively and negatively by temperature.	(Huff et al. 2003)
Circularity	4π x leaf area / $perimeter^2$	The proportional length of leaf margin that is available for interaction with the atmosphere: colder climates should be associated with a greater proportion of margin exposed to the atmosphere and thus low values for circularity.	(Huff et al. 2003)
Dissection	Presence or absence of dissected leaves (teeth, lobes)	Plants growing in cold environments often have more teeth.	(Nobel 1983, Schuepp 1993 and references therein)
Degree of dissection	Deepness of dissection	A more divided leaf will have a smaller boundary layer and better convective heat exchange. Negatively correlated with mean annual temperature.	(Huff et al. 2003, Royer et al. 2005)
Number of teeth	Number of primary and secondary teeth	More teeth per leaf will have a smaller boundary layer and better convective heat exchange. Negatively correlated with mean annual temperature. Associated with water loss, gas exchange, and carbon fixation.	(Huff et al. 2003, Royer et al. 2005)
Standardized tooth count: Number of teeth / perimeter	(mm^{-1})	See previous, corrected for perimeter.	(Royer et al. 2005)
Standardized tooth count: Number of teeth / blade area	(mm^{-2})	See previous, corrected for blade area.	
Shape PC1	Scores on MorphoJ principal component 1 (dimensionless)	Leaf length / width ratio, from roundish short leaves to elongated narrow leaves or needles. Narrow leaves can facilitate cooling by increasing transpiration rates via a thin boundary layer.	(Yates et al. 2010)
Shape PC2	Scores on Shape principal component 2 (dimensionless)	From obovate to elliptical leaf shapes.	
Leaves PC1	Scores on morphology principal component 1 (dimensionless)	Traits related to leaf area and shape, i.e. small leaves, large perimeter / area ratio, elongated narrow leaf shapes, low SLA.	

Leaves PC2	Scores on morphology principal component 2 (dimensionless)	Traits related to leaf complexity, i.e. high complexity and compactness, deeply dissected, non-circular, large #teeth / blade area and #teeth / perimeter, large perimeter and Feret-diameter ratio, and obovate leaf shapes.	
Climate PC1	Scores on climate principal component 1 (dimensionless)	Related to precipitation, i.e. high MAP and high precipitation in wettest, driest, warmest and coldest quarters of the year.	
Climate PC2	Scores on climate principal component 2 (dimensionless)	Related to temperature, i.e. high MAT, and high minimum and maximum temperatures of the coldest and warmest months of the year.	


A.
Average Proteaceae
leaf shape
Principal Component 2
Principal Component 1
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
0.4


B.
MAT
MaxTWarmMonth
MinTColdMonth
√PrecipWettestQ
√PrecipWarmestQ
√AnnualPrecip
√PrecipDriestQ
√PrecipColdestQ
PC2 (28%)
PC1 (54%)
open
closed
both

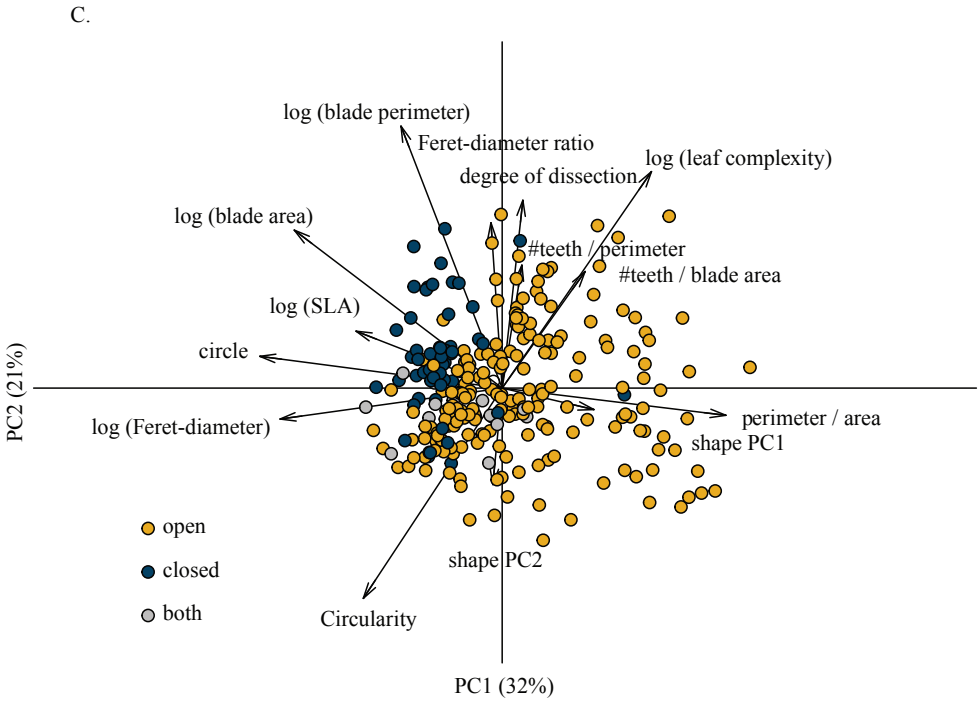


Figure 1
Proteaceae PCAs for leaf shape, climate and morphology. **A.** PCA of leaf shape as from four landmarks in MorphoJ based on 269 Proteaceae species. The leaf shape of extremes on principal component (PC) 1 and PC2 are indicated in black (PC1 -0.5, PC1 0.4; PC2 -0.4, PC2 0.4). PC1 (explaining 59% of the variation in the data) reflects changes in leaf length to width ratio (circular to elongated), and PC2 (39% of the variation) reflects changes in shape from obovate to elliptical. **B.** PCA of climate variables of Proteaceae occurring in open and closed habitats, based on mean climate values for 1643 species. PC1 explains 54% and PC2 and additional 28% of the variation in the data. 'Precip' = precipitation, 'T' = temperature, 'Max' = maximum, 'Min' = minimum, 'Q' = quarter of the year, 'Cold' = coldest, 'Warm' = warmest, 'MAT' = mean annual temperature, 'MAP' = mean annual precipitation. **C.** PCA of leaf morphological traits of Proteaceae occurring in open and closed habitats, based on mean trait values for 269 species. PC1 explains 32% and PC2 an additional 21% of the variation in the data.

Divergent selection in open and closed vegetation types

To understand which factors might drive a faster diversification in open habitats (e.g. Mediterranean Sauquet et al. 2009), we fitted seven likelihood models for continuous trait evolution with increasing complexity performed in the OUwie R package (Beaulieu et al. 2012). We compared the estimated parameters among supported candidate models, performed on each trait and climate variable and on the two major shape, leaf morphological and climatic axes as summarized by PC1 and PC2 (Fig. 1). These seven models and their parameters are described in Table 2 and include variations on the Ornstein-Uhlenbeck model with parameters describing the selective optimum of the trait (θ), the rate of stochastic evolution away from the optimum (σ^2), and the strength of selection towards the optimum (α) (Hansen 1997, Butler and King 2004). These parameters can potentially differ for lineages in open and closed vegetation types (indicated by OU_2 models), suggesting that traits and climatic niches follow distinct evolutionary pathways in these vegetation types. These models were fitted on the Proteaceae MCC tree after assigning nodes in the tree to either open or closed vegetation, based on ancestral state reconstructions (ASR) under the unequal transition rate model using the 'ace' function in the ape R package (Paradis et al. 2004). These reconstructions yielded identical results to ASR under stochastic character mapping. Importantly, some Proteaceae genera are missing from the tree, i.e. *Bellendena, Placospermum, Agastachys, Symphionema, Cenarrhenes, Beaupreopsis*, which may influence the ancestral state reconstructions at the more basal nodes of the tree. We checked model performance and reliability of the parameter estimates and model likelihood, by evaluating the eigenvalues of the models, which should be positive. Possible difficulties in estimating α from the data (often when estimating it jointly with σ^2), such as in the $OU_{2\alpha}$ and $OU_{2\sigma\alpha}$ models, may lead to problematic inference, inflated standard errors around mean parameter estimates, and negative eigenvalues of the Hessian (Beaulieu et al. 2012). In these cases, the model was not considered. We recorded the likelihood of each model and used the Akaike Information Criterion (AIC) to identify the model that best described our data.

Table 2

Likelihood models and their parameters to describe the evolution of leaf functional traits and climatic niches and the association with vegetation types, performed in OUwie. O = open vegetation; C = closed vegetation; #P = number of free parameters in the model, θ = optimum, σ^2 = rate of stochastic evolution, α = strength of selection towards the optimum, BM = Brownian motion, OU = Ornstein–Uhlenbeck.

Model	#P	σ^2_o	σ^2_c	θ_o	θ_c	α_o	α_c	Interpretation
BM	2	$\sigma^2_o = \sigma^2_c$		$\theta_o = \theta_c$		$\alpha = 0$		no association between vegetation types and traits, stochastic trait evolution
BM_σ	3			$\theta_o = \theta_c$		$\alpha = 0$		different rate of stochasticity for trait evolution in open and closed vegetation types
OU_1	3	$\sigma^2_o = \sigma^2_c$		$\theta_o = \theta_c$		$\alpha_o = \alpha_c$		no association between vegetation types and traits, trait evolution towards single optimum
OU_2	4	$\sigma^2_o = \sigma^2_c$				$\alpha_o = \alpha_c$		different optima for trait evolution in open and closed vegetation types
$OU_{2\sigma}$	5					$\alpha_o = \alpha_c$		different optima and rate of stochasticity for trait evolution in open and closed vegetation types
$OU_{2\alpha}$	6	$\sigma^2_o = \sigma^2_c$						different optima and strength of selection towards the optimum for trait evolution in open and closed vegetation types
$OU_{2\sigma\alpha}$	6							different optima, rate of stochasticity, and strength of selection towards the optimum for trait evolution in open and closed vegetation types

Estimating rates of morphological and climatic niche evolution

We analysed the tempo and mode (the 'rate') of functional trait and climatic niche evolution using Bayesian analysis of macro-evolutionary mixtures (BAMM) (**www.bammproject.org**), which analyses complex mixtures of evolutionary processes on phylogenetic trees (Rabosky 2014, Rabosky et al. 2014a). BAMM uses reversible-jump MCMC simulation to identify the number and location of possible transitions between different macro-evolutionary 'regimes' in functional trait and climatic niche evolution on the tree that best explain the distribution of trait and climatic niche values across the tips, under a compound Poisson process model of rate variation, thereby accounting for rate variation through time and among lineages. A 'regime' is a shared dynamic process of trait or niche evolution under Brownian motion of a subset of related lineages on a phylogenetic tree, as some subclades might evolve under faster or slower regimes of trait and / or niche evolution than others. This approach is different from the previous (OUwie) analyses, because in BAMM we do not need to define 'regimes' (e.g. open / closed) a priori, and instead of comparing the fit of several likelihood models, BAMM uses a Bayesian approach to detect heterogeneity in the evolution of a variable on the tree. We ran BAMM on the two major leaf morphological and climatic axes as summarized by PC1 and PC2 (Fig. 1B and 1C). For each variable, BAMM was run for 50 million generations on the MCC tree, and we discarded the first 10% of the generations as burnin. We did not take phylogenetic uncertainty into account, mainly because we are interested in the overall functional trait and climatic rate heterogeneity in the clade – which will be detected on the MCC tree and is not expected to differ substantially between trees – and not in the exact position of shifts in regimes (D. Rabosky, pers. comm.). Convergence of MCMC runs was assessed by computing effective sample sizes for the likelihood of the data and for the number of distinct regimes, ensuring at least 200 independent post-burnin samples from the posterior. We extracted the species specific functional trait and climatic niche rates ('tip-rates') using BAMMtools (Rabosky et al. 2014b), and plotted the variation in rates through time for open and closed forest clades (Fig. 2).

We performed PGLS to test if log-transformed tip-rates of morphological and climatic niche evolution were correlated. Although this phylogenetic error-structure does not reflect the compound Poisson process model used in BAMM, we consider this a reasonable solution to account for autocorrelation in rates across the tree (D. Rabosky, pers. comm., Huang and Rabosky 2014). To evaluate the type I error rates (incorrect rejection of a true null hypothesis of no correlation) when regressing the trait rates onto the climatic niche rates (i.e. a correlation could be found simply because of autocorrelation between the nodes in the tree), we simulated a neutral trait under Brownian motion ($\sigma^2 = 0.1$) using the fastBM function in the 'phytools' R package (Revell 2012) on the MCC tree 100 times, and performed a PGLS on this trait as the response variable and our climatic tip-rates as explanatory variables. These results showed that our test correctly failed to reject the true null hypothesis of no correlation (in this example) in 95% of the cases (results not shown). In addition, to assess if open vegetation lineages show faster rates of leaf morphological and climatic niche evolution than closed vegetation lineages, we performed a phylogenetic anova using the 'aov.phylo' function in the geiger R package (Harmon et al. 2008), using the morphological and climatic tip-rates as response variables and vegetation type (open/closed/both) as explanatory variable.

Results

The major axes of morphology and climate tolerance

Shape PC1 and shape PC2 accounted for 59% and 39% of the variation in the data respectively. Shape PC1 mainly reflects changes in leaf length to width ratio (circular to elongated), and shape PC2 mainly reflects changes in shape from obovate to elliptical (Table 1, Fig. 1A). Climate PC1 explained 54% and climate PC2 28% of the variation in the data. Climate PC1 is most strongly related to

precipitation and climate PC2 to temperature. Species in open and closed vegetation types occupy different dimensions in climate space (Table 1, Fig. 1B). The PCA based on the morphological traits indicates that leaves PC1 explained 32% and leaves PC2 an additional 21% of the variation in the leaf morphological data. Leaves PC1 is associated to traits related to blade area, shape and sclerophylly and leaves PC2 is associated to complexity of the leaf, i.e. dissection. Closed vegetation species have predominantly negative scores on leaves PC1, and predominantly positive scores on leaves PC2, and open vegetation species occupy most of the remaining trait space (Table 1, Fig. 1C).

Phylogeny

We obtained a Proteaceae phylogeny and divergence times for clades (Fig. 2, Table S3, MCC tree will be deposited in the Dryad Digital Repository). Consistent with the analysis of Sauquet et al. (2009), divergence time estimates suggest that Proteaceae started diversifying 96.1–115.5 Ma, most subfamilies 45.7–90.1 Ma, tribes 43.1–78.7 Ma and subtribes 21.1–65.6 Ma (Table S2). There is substantial uncertainty in the absolute timing of divergences, indicated by relatively large 95% HPDs around the nodal age estimates (Table S3).

The effect of climate on trait variation

There was a strong effect of climate on functional traits. PGLS indicated that both climate PC1 and PC2 explained, after correcting for the phylogenetic dependence of data points, significant proportions (on average 17% and 6% respectively) of the variation in leaf traits. Specifically, climate PC1 (i.e. positive scores associated with precipitation) was correlated with circular, large, high SLA leaves, with a small degree of dissection, a small perimeter/area and a small number of teeth / blade area, and thus a low leaf complexity. Furthermore PC1 was associated with negative scores on leaves PC1 (Table 3, Fig. S1). Climate PC2 (i.e. positive scores associated with temperature) was associated with large, non-circular leaves with a high Feret-diameter ratio (i.e. large and/or narrow leaves), and negative scores on leaves PC2 and shape PC2 (i.e. obovate leaf shapes) (Table 3, Fig. S2).

Table 3
Phylogenetic generalized least squares (PGLS) regression models of Proteaceae species mean functional traits as a response to climate principal component (PC) axes, based on 216 species. Lambda (λ) refers to phylogenetic signal in the model residuals based on the transformation structure of the covariance matrix (if low λ =0, if high λ=1). The adjusted R^2 refers to total variation in response variable explained by the explanatory variables (between 0 and 1). P = p-value, n.s. = not significant.

Trait	Transformation structure (residuals)	P of $\lambda \neq 0$	Effect of climate PC1 or PC2?	Estimate	P	Adj. R^2
Shape PC1	λ = 0.572	**< 0.0001**	n.s.			
Shape PC2	λ = 0.465	**< 0.0001**	PC2	-0.157	**<0.046**	0.01
Leaves PC1	λ = 0.423	**< 0.0001**	PC1	-0.573	**<0.0001**	0.146
Leaves PC2	λ = 0.354	**< 0.0001**	PC2	-0.229	**0.005**	0.027
Log (blade area)	λ = 0.470	**< 0.0001**	PC1, PC2	0.392, 0.174	**<0.0001, 0.018**	0.174
Log (effective leaf area)	λ = 0.460	**< 0.0001**	PC1	0.628	**<0.0001**	0.172
Log (SLA)	λ = 0.336	**< 0.0001**	PC1	-0.1	**<0.0001**	0.064
Log (blade perimeter)	λ = 0.345	**< 0.0001**	PC1, PC2	0.101, 0.133	**0.018, 0.003**	0.082
Circularity	λ = 0.318	**< 0.0001**	PC1, PC2	0.03, -0.021	**0.003, 0.04**	0.038
Log (Feret-diameter)	λ = 0.325	**0.0002**	PC1	0.247	**<0.0001**	0.109
Log (Complexity)	λ = 0.213	**<0.0001**	PC1	-0.094	**0.0001**	0.061
Log (Dissection)	λ = 0.724	**< 0.0001**	PC1	-0.202	**0.002**	0.112
Log (Feret-diameter ratio)	λ = 0.038	**<0.0001**	PC2	0.113	**0.005**	0.029
Log (Perimeter / area)	λ = 0.444	**< 0.0001**	PC1	-0.29	**<0.0001**	0.18
Log (Teeth / perimeter)	λ = 0.347	**< 0.0001**	n.s.			
Log (Teeth / blade area)	λ = 0.187	**<0.0001**	PC1	-0.278	**0.0001**	0.181

Divergent selection in open and closed vegetation types
Different selection pressures appear to act in open (predominantly Mediterranean) and closed (predominantly tropical rainforest) vegetation types. For most trait and climate variables the $OU_{2\sigma}$ model, with optima defined by open and closed vegetation and a different rate of stochastic evolution away from the optimum in open and closed vegetation, was strongly favoured over the other models, indicated by the ΔAICc and the Akaike Weight, which reflect the relative likelihood of this model compared to the other models (Table S4). However, for some traits a simpler model was favoured (e.g. OU for #teeth, OU_2 for effective leaf area and traits related to dissection), or a more complex model (e.g. $OU_{2\sigma\alpha}$ for perimeter / area, Table S4).

The models suggest that compared to closed vegetation, open vegetation selects for smaller, less circular, lower SLA leaves, with a higher leaf compactness, leaf complexity, and perimeter / area, a higher degree of dissection, and more teeth / perimeter or blade area, and a smaller Feret-diameter ratio (i.e. closed vegetation leaves are either bigger and/or narrower than open vegetation leaves) (Table 4). Furthermore, climatic tolerance evolved towards lower temperatures (MAT, MinTColdMonth and MaxTWarmMonth) and less precipitation (PrecipWettestQ, PrecipDriestQ, PrecipWarmestQ and MAP) in open compared to closed vegetation Proteaceae. Interestingly, the rate of stochastic evolution away from the optimum (σ^2) was found to be greater in open vegetation than in closed vegetation for most variables for which the $OU_{2\sigma}$ or the $OU_{2\sigma\alpha}$ model was found to be the best fit given the data (Table 4).

Table 4

Model estimates for the preferred model of evolution for each variable (climatic niche and functional traits) in Proteaceae, based on 216 species (model selection in Table S4). O = open vegetation, C = closed vegetation, θ = selective optimum, α = the strength of selection towards θ, σ² = the rate of stochastic evolution away from θ, se = standard error of θ. In case open and closed vegetation were not different for a variable, given the model, the estimate is reported in the first column (i.e. αO, σ²O, θO and θO se), but can be applied to the respective subsequent column (i.e. αC, σ²C, θC and θC se).

Variable	Model	θ O	θ C	α O	α C	σ² O	σ² C	θ O se	θ C se
Log (blade area)	$OU_{2\sigma}$	6.147	8.39	0.082		0.415	0.077	0.155	0.124
Log (effective leaf area)	OU_{2}	4.179	7.19	1.191		9.644		0.154	0.34
Log (SLA)	$OU_{2\sigma}$	1.355	1.936	0.529		0.301	0.082	0.042	0.047
Log (blade perimeter)	$OU_{2\sigma}$	5.279	6.031	0.357		0.611	0.152	0.074	0.079
Circularity	$OU_{2\sigma}$	0.278	0.33	2.275		0.219	0.084	0.017	0.023
Log (Feret-diameter)	$OU_{2\sigma}$	2.099	2.856	1.357		3.043	0.66	0.081	0.083
Log (Compactness)	$OU_{2\sigma}$	4.3	3.725	3.28		7.769	1.149	0.083	0.071
Log (Complexity)	$OU_{2\sigma}$	2.159	1.867	3.182		1.891	0.279	0.041	0.035
Log (Dissection)	OU_{2}	-1.319	-2.101	3.313		3.425		0.092	0.2
Log (Feret-diameter ratio)	$OU_{2\sigma}$	1.159	1.431	3.310		4.363	2.186	0.061	0.097
Log (Perimeter / area)	$OU_{2\sigma\alpha}$	-0.916	-2.324	0.114	0.118	0.291	0.025	0.091	0.059
Log (Teeth / perimeter)	OU_{2}	-2.919	-3.323	3.313		4.433		0.096	0.227
Log (Teeth / blade area)	OU_{2}	-4.068	-5.598	3.281		7.153		0.123	0.29
Shape PC1	$OU_{2\sigma}$	0.023	-0.029	3.311		0.269	0.098	0.015	0.021
Shape PC2	$OU_{2\sigma}$	0.003	0.027	3.311		0.169	0.062	0.012	0.016
Leaves PC1	$OU_{2\sigma}$	0.611	-1.842	2.504		23.62	1.625	0.166	0.096
Leaves PC2	$OU_{2\sigma}$	-0.186	0.346	3.156		20.04	6.761	0.136	0.175
Climate PC1	$OU_{2\sigma}$	-0.268	3.372	3.307		7.376	13.35	0.081	0.255
Climate PC2	OU_{2}	-0.48	0.991	2.74		8.051		0.093	0.218
MAT	OU_{2}	15.8	20.5	0.86		14.285		0.18	0.424
MinTColdMonth	$OU_{2\sigma}$	5.872	12.44	0.147		2.472	6.155	0.251	0.826
MaxTWarmMonth	$OU_{2\sigma\alpha}$	26.464	27.657	0.114	0.107	2.064	1.744	0.28	0.425
√ (PrecipWettestQ)	$OU_{2\sigma}$	17.87	31.68	3.293		101.4	271.8	0.301	1.154
√ (PrecipDriestQ)	OU_{2}	9.347	13.18	1.07		14.98		0.206	0.475
√ (PrecipColdestQ)	OU	15.23		3.299		79.349		0.245	
√ (PrecipWarmestQ)	$OU_{2\alpha}$	11.64	28.33	0.087	0.091	6.611		0.527	1.109
√ (MAP)	$OU_{2\sigma\alpha}$	11.62	29.56	0.099	0.103	7.889	4.039	0.505	0.847

Rates of morphological and climatic niche evolution

We detected a significant positive effect of the macro-evolutionary climatic niche rates on the rates of functional trait evolution in Proteaceae (species tip-rates resulting from BAMM), after correcting for the phylogenetic dependence of data points, with the exception of the effect of climate PC1 on leaves PC2 (PGLS for leaves PC1 rate as response variable: $\lambda=1$, $\kappa=1.6$, $P<0.05$ for climate PC1 rate, and $P<0.0001$ for climate PC2 rate [climate PC rates as explanatory variables]; for leaves PC2 rate as response variable: $\lambda=1$, $\delta=0.24$, $P=0.28$ for climate PC1 rate, and $P<0.0001$ for climate PC2 rate [climate PC rates as explanatory variables]) (Fig. 3). This result indicates that species which show faster climatic niche evolution also diversify faster morphologically (Fig. 3). Furthermore, these functional trait and climatic niche rates are different for open and closed vegetation clades (phylogenetic ANOVA climate PC1 rate as response variable: $F=13.84$, $p<0.001$; climate PC2 rate as response variable: $F=40.72$, $p<0.0001$; leaves PC1 rate as response variable: $F=11.05$, $p<0.01$; leaves PC2 rate as response variable: $F=28$, $p<0.0001$). Specifically, open vegetation clades show significant higher rates of niche and functional trait evolution. Furthermore, faster rates of climatic niche and functional trait evolution over time were observed in open vegetation clades, whereas closed vegetation clades showed predominantly constant rates over time (Figs. 2 and 4).

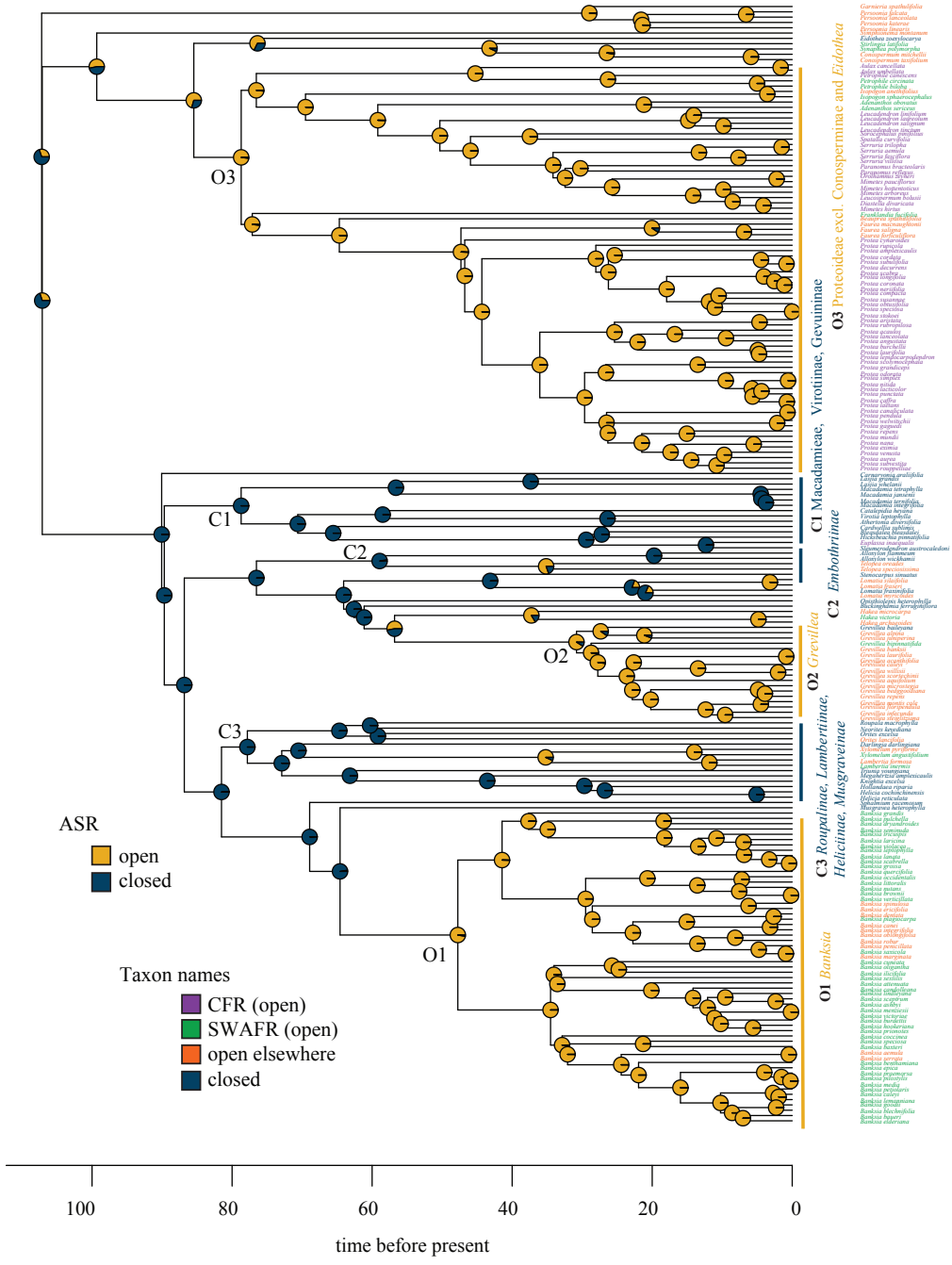


Figure 2

Proteaceae maximum clade credibility tree inferred using BEAST, with ancestral state reconstructions for vegetation type (open / closed) under an unequal transition rate model (yellow = open and blue = closed vegetation). Open (O1-O3) and closed (C1-C3) vegetation clades are defined based on the ancestral state, which should have a probability of >0.9 to be present in a certain vegetation type. At least five independent shifts between vegetation types are inferred. Taxon names are coloured based on their occurrence in the Cape Floristic Region (purple), the Southwest Australian Floristic Region (green), open non-Mediterranean (red) and closed vegetation (blue).

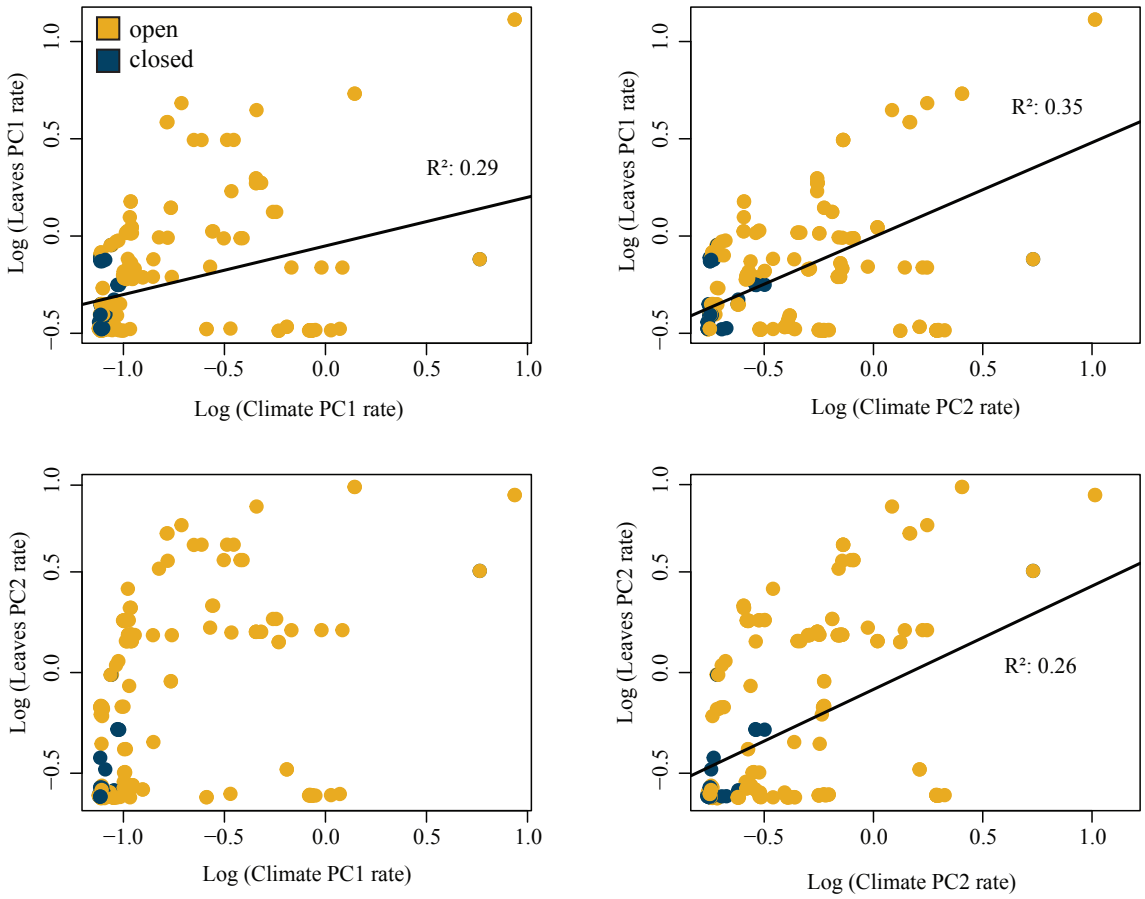


Figure 3
The rate of evolution in leaf morphology (disparity) plotted against the rate of climatic niche evolution, taken from species tip data resulting from BAMM ('tip-rates'). PGLS indicates a strong effect of the variation in climatic niche rate on the rate of morphological evolution in all cases except from climate PC1 on leaves PC2, and species in open vegetation (yellow) generally have higher rates than species in closed vegetation (blue). The R^2 (between 0 and 1) refers to the total amount of variation explained in the data.

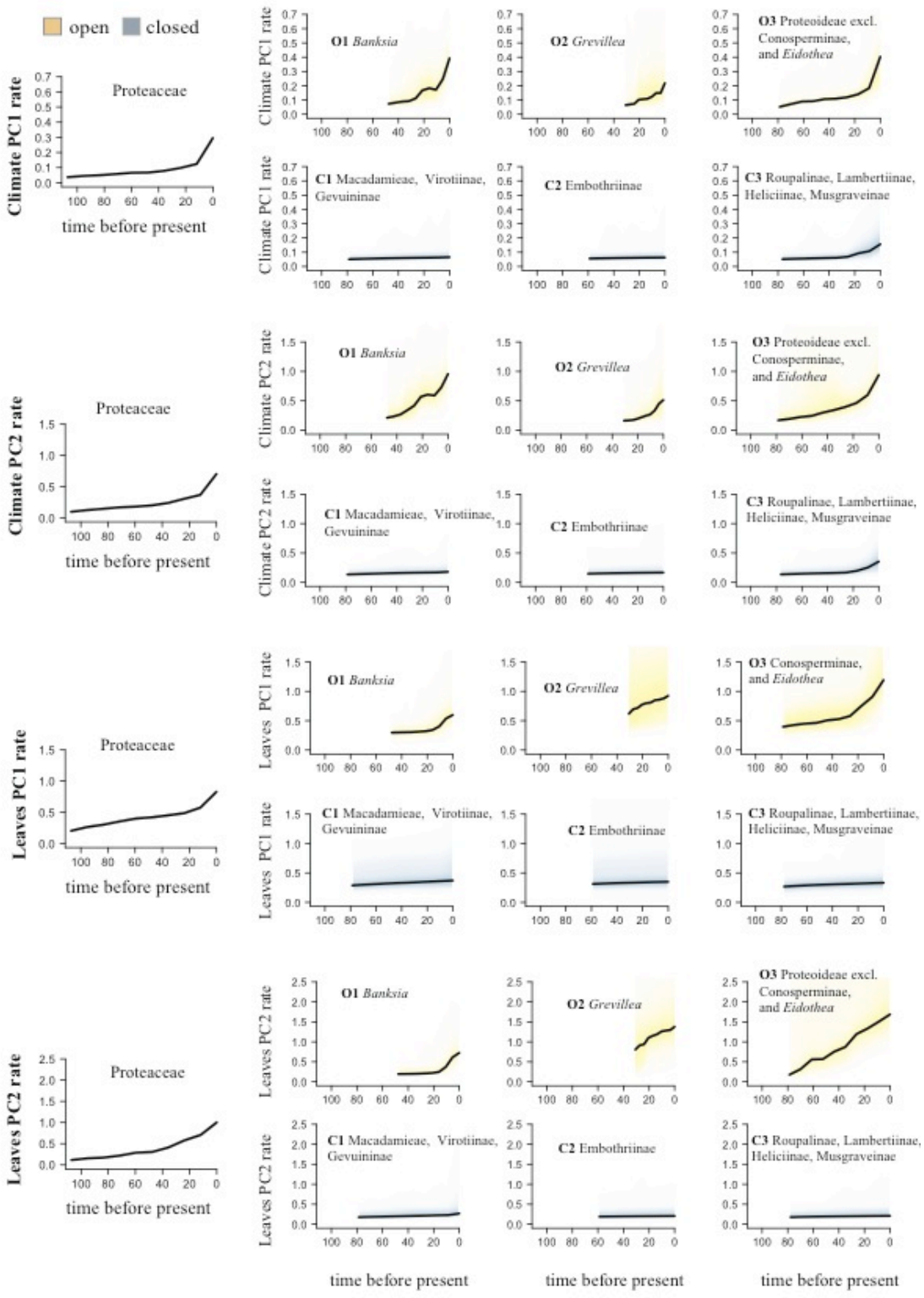


Figure 4
Rates through time plots for climatic niche (climate PC1 and PC2) and leaf morphology (leaves PC1 and PC2) for Proteaceae clades resulting from BAMM. The clades indicated in Fig. 2 are shown. Open vegetation clades (yellow, O1 – O3) generally show faster inferred rates of climatic niche and functional trait evolution than closed vegetation clades (blue, C1 – C3).

Discussion

We demonstrate that the variation in Proteaceae functional traits is partly driven by present-day climate (Table 3) as well as stabilizing selection towards ecological optimal and divergent leaf designs in open (predominantly CFR and SWAFR Mediterranean) and closed (predominantly rainforest) vegetation types (Table 4). Furthermore, we show that the evolutionary rates for functional traits and climatic niches in Proteaceae are correlated (Fig. 3), and that these rates are particularly high in open vegetation clades (Figs. 3 and 4). These results suggest that selection for divergent trait optima in open and closed vegetation types may create morphologically distinct floras. Furthermore, the macro-evolutionary rates of functional trait and climatic niche evolution may be dependent on the system.

Functional traits and the evolution of climate

We show that leaf traits are correlated with climate variation, as is generally expected (Reich et al. 2003). After correcting for phylogeny, between 4% and 35% of the variation in leaf functional traits in Proteaceae is explained by climate (Table 3, Fig. S2). The largest part of the variation remains unexplained, and may be correlated to factors other than climate, such as herbivory (Coley 1983), soil nutrient status (Cunningham et al. 1999), whole-plant trade-offs and allometric constraints (Wright et al. 2004). Although links between traits and vegetation types and / or climates has previously been shown in Proteaceae (Thuiller et al. 2004, Jordan et al. 2005, Jordan et al. 2008, Yates et al. 2010, Mitchell et al. 2015), we show here that divergent selective optima for Proteaceae traits are dependent on the - supposedly different - selection pressures by the vegetation type a lineage has evolved in (Table 4). Corroborating these results, Jordan et al. (2015) showed that stomatal size and genome size in Proteaceae were significantly different between open and closed vegetation. These results are expected, as these vegetation types are mostly geographically separated and have had different climate regimes during the Cenozoic, thereby selecting for different traits during the *in-situ* diversification of Proteaceae lineages in the several regions (e.g. closed forests in Queensland and New Guinea, open shrublands in the CFR, SWAFR and eastern Australia).

The expansion and contraction of these vegetation types over evolutionary time may have influenced the adaptive landscapes and 'ecological' opportunities provided by these systems. During Miocene (ca. 23–5 Ma) climate change the global climate became cooler, drier, and more seasonal, leading to the origin and expansion of open arid and alpine biomes and the contraction of Australian rainforests, typical for the closed forest Proteaceae, to a small fraction of their original extent (Martin 2006, Byrne et al. 2008, Crisp and Cook 2013). Mediterranean-type ecosystems in the CFR and the SWAFR may have emerged from the Miocene onward (Hopper and Gioia 2004, Martin 2006, Cowling et al. 2009, Dupont et al. 2011). These opportunities may consequently have influenced the rates of evolutionary change in morphology and climatic niches in open and closed vegetation lineages. Indeed, there seems to be an increase in climatic niche and morphological rates from ~20 Ma till present in open vegetation Proteaceae clades (Fig. 4). Thus, the deep evolutionary history of Proteaceae lineages in open and closed vegetation types (Fig. 2), and the dynamic history of these vegetation types, has certainly influenced Proteaceae speciation and extinction rates, the evolution of leaf traits, and the colonization of climatic niches within these vegetation types.

Disparification rate depends on vegetation and climatic rate

The higher rate of stochastic evolution (σ^2) of traits and climatic niches away from the ecological optimum in open compared to closed vegetation Proteaceae (Table 4) suggests that the variance of these variables in open vegetation may be higher than in closed vegetation, leading to more variation at the tips of the phylogeny (Beaulieu et al. 2012). This result is consistent with variable rates of both

functional trait and climatic niche evolution over time in open and closed vegetation Proteaceae lineages (Fig. 4), suggesting overall stronger niche conservatism in closed vegetation clades (low rates of disparification and niche divergence, Figs. 3 and 4), and relatively higher niche lability and divergence (disparification) in open vegetation clades (high rates of disparification and niche divergence, Figs. 3 and 4) (Kozak and Wiens 2010). We show that, comparable to high diversification rates of clades associated with open habitats (annuals, herbs) (Smith and Donoghue 2008a), (morphological) disparification and climatic niche rates are also higher in open than in closed vegetation systems (Figs. 3 and 4). Furthermore, more generally we find that rates of climatic niche and functional trait evolution are strongly correlated in Proteaceae (Fig. 3). This may reflect the dependence of morphological evolution and adaptation on climatic evolution, or conversely, the morphology allows the colonization of new climatic niches (pre-adaptation). Fast rates of climatic niche evolution in open habitats was previously shown for *Pelargonium* (Martínez-Cabrera et al. 2012) and *Babiana* (Iridaceae) (Schnitzler et al. 2012) in the CFR. *Pelargonium* was also shown to have a great morphological variation (Jones et al. 2009), particularly in leaf shapes, but the link to rates of climatic niche evolution were not tested (but see Mitchell et al. 2015).

Diversity in climatically buffered and non-buffered habitats

We hypothesize that in open vegetation, for the same spatial climatic pattern, plants may experience a greater range of climates than if the same area was covered in forest, due to climatic buffering under a forest canopy. Indeed, under forest cover the extremes in the climate regime are flattened (Chen et al. 1999, Clinton 2003, Linder et al. 2012). This may increase the exposure to diversifying (disruptive) selection in open compared to closed systems, which may consequently affect diversification rates as lineages may track these climatic 'opportunities'. Indeed, (adaptive) ecological divergence was previously shown in the white protea clade (Proteaceae) in the CFR (Carlson et al. 2011). Carlson et al. (2011) showed that leaf size, leaf shape and SLA differences were associated with gradients in rainfall seasonality, drought stress, cold stress (and less frequently soil fertility), suggesting that plant populations in the CFR differentiate adaptively, which may lead to speciation. Furthermore, higher net diversification rates (due to increased speciation rates, Reyes et al. 2015) were detected for Proteaceae in open Mediterranean-type ecosystems (Sauquet et al. 2009). Our results thus suggest that climatic niche evolution and morphological evolution may be linked to diversification rates (Ricklefs 2004, Kozak and Wiens 2010, Rabosky et al. 2013), and the micro-evolutionary pattern of ecological (morphologically- and climatically-driven) divergence in the white proteas (Carlson et al. 2011) may be more generally applicable to Proteaceae.

However, climatic heterogeneity may not be the only trigger for diversifying selection in these systems, as the climatic heterogeneity differs substantially between open systems, and in particular when comparing the CFR to the SWAFR (Jiménez and Ricklefs 2014, Litsios et al. 2014). The sandplains of the SWAFR are climatically much more homogeneous than the micro-climatic niches in the CFR, and the morphological disparification in the SWAFR may thus not exclusively result from climatic variation (Fig. 3). This suggests that our results are probably not sufficient to explain the parallel radiations of Proteaceae in open systems. Proteaceae in the SWAFR are more species-rich than in the CFR, and this has resulted in the SWAFR from a mix of fast and medium diversification rates, whereas diversification rates in the CFR are mostly fast (Sauquet et al. 2009). Additional factors, such as climatic and topographical stability of the system over time (Cowling et al. 2014), or factors related to other components of habitat heterogeneity, such as edaphic conditions, fire and microhabitats (Linder 2003, Hopper and Gioia 2004), or selective pressure by herbivores, dispersers or pollinators, may thus have additionally influenced diversification and species-richness in the SWAFR and the CFR (Thuiller et al. 2006). Furthermore, the SWAFR flora is less isolated, and diversity has less resulted from *in-situ* diversification (i.e. due to more lineages with sister groups

outside the region) than in the CFR (Sauquet et al. 2009). This may indicate that speciation in open habitats in Australia may also occur through geographic isolation, which may 'accidently' correlate to climatic factors which may impose selection on morphology, i.e. leaf form.

Nevertheless, our study is the first to our knowledge to directly link rates of morphological evolution to climatic niche evolution at a macro-evolutionary scale (Ackerly 2009), and emphasizes the contribution of strong trait-environment associations (Mitchell et al. 2015) to understanding the causes of evolutionary radiations in open Mediterranean-type ecosystems.

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Supporting Information Chapter V

Table S1: GenBank accession numbers for Proteaceae (and outgroup) sequences used in this study.

Taxon	*mat*K	*atp*B	*rbc*L	*rpl*16	*trn*-L exon	*trn*L-F spacer
Acidonia microcarpa			gi188529286			
Adenanthos obovatus		gi3850927	gi4098529			
Adenanthos sericeus	gi166156234		gi133930656			
Agastachys odorata	gi166156236	gi3850905	gi133930634			
Alloxylon wickhamii		gi3850975				
Alloxylon flammeum	gi166156238		gi133930698			
Athertonia diversifolia	gi166156240	gi194267365	gi188529288			
Aulax cancellata			gi125991692			
Aulax umbellata	gi166156241				gi188529210	gi188529214
Austromuellera trinervia	gi56131331	gi3850947	gi133930716		gi56131346	gi56131351
Banksia aculeata				gi24181704		
Banksia aemula	gi56131267	gi62184015		gi24181708	gi24181612	gi24181657
Banksia ashbyi	gi56131285	gi62184031		gi24181721	gi24181621	gi24181666
Banksia attenuata	gi56131283	gi62184029		gi24181720	gi24181620	gi24181665
Banksia audax				gi24181735		
Banksia baueri	gi56131279	gi62184027		gi24181718	gi24181618	gi24181663
Banksia baxteri	gi56131273	gi62184021		gi24181711	gi24181615	gi24181660
Banksia benthamiana	gi56131293	gi62184037		gi24181734	gi24181625	gi24181670
Banksia bipinnatifida	gi56131325	gi62184069		gi56131339	gi56131344	gi56131349
Banksia blechnifolia				gi24181726		
Banksia brevidentata				gi24181728		
Banksia brownii	gi56131301	gi62184045		gi24181757	gi24181629	gi24181674
Banksia burdettii				gi24181714		
Banksia caleyi				gi24181703		
Banksia calophylla	gi56131317	gi62184061		gi24181783	gi24181637	gi24181682
Banksia candolleana	gi56131269	gi62184017		gi24181709	gi24181613	gi24181658
Banksia canei				gi24181745		
Banksia chamaephyton				gi24181725		
Banksia coccinea	gi56131287	gi62184033		gi24181722	gi24181622	gi24181667
Banksia cuneata	gi56131257	gi3850945		gi24181699	gi24181608	gi24181653
Banksia dentata				gi24181742		
Banksia dolichostyla				gi24181767		
Banksia drummondii			gi4098537			
Banksia dryandroides	gi56131305	gi62184049		gi24181763	gi24181631	gi24181676
Banksia elderiana	gi56131261	gi62184009		gi24181701	gi24181610	gi24181655
Banksia elegans	gi56131259	gi62184007		gi24181700	gi24181609	gi24181654
Banksia epica				gi24181732		
Banksia ericifolia	gi56131299	gi62184043	gi133930672	gi24181755	gi24181628	gi24181673
Banksia falcata	gi56131327	gi62184071		gi56131340	gi56131345	gi56131350
Banksia foliosissima	gi56131313	gi62184057		gi24181781	gi24181635	gi24181680
Banksia formosa	gi56131323	gi62184067		gi56131338	gi56131343	gi56131348
Banksia grandis	gi56131295	gi62184039		gi24181737	gi24181626	gi24181671
Banksia goodii				gi24181729		
Banksia grossa				gi24181766		
Banksia heliantha			gi188529294			
Banksia hiemalis				gi24181727		
Banksia hookeriana				gi24181716		

Banksia ilicifolia	gi56131255	gi62184003		gi24181697	gi24181607	gi24181652
Banksia incana				gi24181778		
Banksia integrifolia				gi24181750		
Banksia laevigata				gi24181736		
Banksia lanata				gi24181775		
Banksia laricina				gi24181777		
Banksia lemanniana				AF482200		
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Banksia lullfitzii	gi56131281			gi24181719	gi24181619	gi24181664
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Banksia nutans	gi56131307	gi62184051		gi24181764	gi24181632	gi24181677
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Banksia oreophila				gi24181762		
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Banksia penicillata				gi24181744		
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Banksia scabrella				gi24181772		
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Banksia speciosa	gi56131275	gi62184023		gi24181712	gi24181616	gi24181661
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Banksia violacea				gi24181776		
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Beauprea spathulifolia	gi166156245					
Beaupreopsis paniculata			gi194293216		gi188529211	gi188529215
Bellendena montana	gi166156247	gi3850899	gi4098531			

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Catalepidia heyana	gi193957831	gi194267367	gi188529290			
Cenarrhenes nitida		gi3850911	gi133930640			
Conospermum mitchellii		gi3850915	gi133930644			
Conospermum taxifolium	gi166156254		gi73811174			
Darlingia darlingiana	gi166156256		gi188529292			
Diastella divaricata	gi166156258		gi188529241			
Diastella parilis			gi125991694		gi188529212	
Dilobeia thouarsii	gi166156260		gi194293218			
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Faurea macnaughtonii					gi56561611	gi56710759
Faurea rochetiana	gi167890116		gi167891347		gi56561614	gi56710760
Faurea rubriflora					gi56561615	gi56710761
Faurea saligna	gi167890126		gi188529243		gi56561612	gi56710762
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Grevillea alpina						gi251766098
Grevillea angustiloba						gi251766104
Grevillea aquifolium			gi133930710			gi251766109
Grevillea baileyana		AF060434				
Grevillea banksii	AF542583				gi121491121	
Grevillea bedggoodiana						gi251766117
Grevillea bipinnatifida						gi251766103
Grevillea caleyi	EU642709	EU642739				
Grevillea curviloba	gi166156277					
Grevillea dilatata						gi251766102
Grevillea dryophylla						gi251766128
Grevillea floripendula						gi251766126
Grevillea ilicifolia						gi251766105
Grevillea infecunda						gi251766132
Grevillea juniperina	gi166156279					
Grevillea laurifolia						gi251766095
Grevillea microstegia						gi251766115
Grevillea montis-cole						gi251766129
Grevillea obtecta						gi251766110

Grevillea renwickiana						gi251766099
Grevillea repens						gi251766100
Grevillea robusta	FJ626529		AF193973		FJ626569	
Grevillea scortechinii						gi251766097
Grevillea steiglitziana						gi251766127
Grevillea willisii						gi251766096
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Hakea myrtoides			HMU79170			
Hakea archaeoides			EU676114			
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Helicia reticulata	gi331704692		gi331704355			
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Heliciopsis lobata					gi188529226	gi188529231
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Isopogon latifolius			gi4098545			
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Lasjia whelanii	gi193957835	gi194267370				
Lasjia hildebrandii	gi166156308					
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Leucadendron gandogeri					gi268526663	
Leucadendron laureolum			gi4098547			
Leucadendron linifolium	gi166156298					
Leucadendron salignum		gi3850929				
Leucadendron tinctum			gi133930658			
Leucospermum bolusii			gi125991696			
Leucospermum pedunculatum	gi166156302					
Lomatia fraseri	gi166156304					
Lomatia myricoides		gi3850979				
Lomatia silaifolia	gi166156306		gi4098549	gi24181791	gi24181645	gi24181690
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Macadamia ternifolia	gi193957841	gi194267376	gi4098551			
Macadamia tetraphylla	gi193957839	gi194267374				
Macadamia integrifolia	gi56131335	gi62184079		gi24181788		gi24181687
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Megahertzia amplexicaulis			gi188529308			
Mimetes arboreus	gi246655189		gi240253208			
Mimetes hottentoticus			gi188529245			
Mimetes pauciflorus			gi125858758			

Mimetes hirtus	gi246655190		gi240253210			
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Orites lancifolia		gi3850943		gi24181790	gi24181644	gi24181689
Orites diversifolia	gi193957803	gi194267340				
Orites myrtoidea			gi133930670			
Orothamnus zeyheri			gi1045643			
Panopsis ferruginea		gi3850961				
Panopsis cinnamomea	gi193957847	gi194267382	gi133930686			
Panopsis pearcei	gi166156320					
Panopsis yolombo	gi166156321					
Paranomus bracteolaris	gi166156323					
Paranomus reflexus			gi188529247			
Paranomus dispersus			gi125858787			
Persoonia katerae	gi41393759	gi6467932				
Persoonia lanceolata			gi4098555			
Persoonia linearis	gi166156325					
Persoonia falcata			gi188529249			
Petrophile canescens	gi146188902					
Petrophile circinata		gi3850921				
Petrophile biloba			gi133930650			
Protea acaulos					gi56561597	gi56710744
Protea acuminata					gi56561528	gi56710697
Protea amplexicaulis					gi56561529	gi56710699
Protea angolensis					gi56561606	gi56710754
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Protea aristata					gi56561511	gi56710676
Protea aspera					gi56561501	gi56710680
Protea aurea					gi56561598	gi56710745
Protea burchellii					gi56561531	gi56710700
Protea caespitosa					gi56561600	gi56710747
Protea caffra					gi56561544	gi56710713
Protea canaliculata					gi56561520	gi56710689
Protea compacta					gi56561554	gi56710722
Protea comptonii					gi56561593	gi56710740
Protea convexa					gi56561555	gi56710724
Protea cordata					gi56561553	gi56710723
Protea coronata					gi56561541	gi56710710
Protea cryophila					gi56561532	gi56710701
Protea curvata					gi56561516	gi56710685
Protea cynaroides	gi166156332	gi164708509	gi133930660		gi56561512	gi56710681
Protea decurrens					gi56561542	gi56710711
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Protea effusa					gi56561502	gi56710670
Protea enervis					gi56561507	gi56710675
Protea eximia					gi56561522	gi56710691
Protea foliosa					gi56561505	gi56710673
Protea gaguedi					gi56561596	gi56710743

Protea glabra					gi56561546	gi56710715
Protea grandiceps					gi56561594	gi56710741
Protea holosericea					gi56561509	gi56710678
Protea humiflora					gi56561583	gi56710730
Protea inopina					gi56561585	gi56710732
Protea intonsa					gi56561558	gi56710727
Protea inyanganiensis					gi56561608	gi56710755
Protea lacticolor					gi56561521	gi56710690
Protea laetans					gi56561609	gi56710756
Protea laevis					gi56561581	gi56710728
Protea lanceolata					gi56561601	gi56710748
Protea laurifolia	gi71891434				gi56561586	gi56710733
Protea lepidocarpodendron					gi56561533	gi56710702
Protea longifolia					gi56561547	gi56710716
Protea lorea					gi56561582	gi56710729
Protea lorifolia					gi56561589	gi56710736
Protea magnifica					gi56561584	gi56710731
Protea montana					gi56561556	gi56710725
Protea mucronifolia					gi56561534	gi56710703
Protea mundii					gi56561517	gi56710686
Protea namaquana					gi56561535	gi56710704
Protea nana					gi56561536	gi56710705
Protea neriifolia	gi166156334				gi56561587	gi56710734
Protea nitida					gi56561595	gi56710742
Protea nubigena					gi56561588	gi56710735
Protea obtusifolia					gi56561548	gi56710717
Protea odorata					gi56561590	gi56710737
Protea parvula					gi56561539	gi56710708
Protea pendula					gi56561519	gi56710688
Protea petiolaris					gi56561605	gi56710752
Protea piscina					gi56561540	gi56710709
Protea pityphylla					gi56561537	gi56710706
Protea pruinosa					gi56561524	gi56710693
Protea pudens					gi56561549	gi56710718
Protea punctata					gi56561525	gi56710695
Protea recondita					gi56561591	gi56710738
Protea repens			gi4098557		gi56561550	gi56710719
Protea restionifolia					gi56561518	gi56710687
Protea revoluta					gi56561526	gi56710694
Protea rouppelliae					gi56561551	gi56710720
Protea rubropilosa					gi56561602	gi56710749
Protea rupicola					gi56561508	gi56710677
Protea scabra					gi56561610	gi56710757
Protea scabriuscula					gi56561506	gi56710674
Protea scolopendriifolia					gi56561515	gi56710684
Protea scolymocephala					gi56561599	gi56710746
Protea scorzonerifolia					gi56561510	gi56710679
Protea simplex					gi56561557	gi56710726
Protea speciosa					gi56561592	gi56710739
Protea stokoei					gi56561523	gi56710692
Protea subulifolia					gi56561552	gi56710721
Protea subvestita					gi56561504	gi56710672

Protea sulphurea	AM889742				gi56561603	gi56710750
Protea susannae	gi166156336				gi56561513	gi56710682
Protea tenax					gi56561503	gi56710671
Protea venusta					gi56561514	gi56710683
Protea vogtsiae					gi56561538	gi56710707
Protea welwitschii					gi56561543	gi56710712
Protea wentzeliana					gi56561604	gi56710751
Protea witzenbergiana	gi246655222		gi240253294		gi56561527	gi56710696
Roupala loxensis	gi273415768					
Roupala merophylla			gi4098559			
Roupala macrophylla		gi3850951	gi6017866			
Roupala monosperma			gi188529221			
Roupala montana	gi193957799	gi194267336		gi24181792	gi24181646	gi24181691
Serruria trilopha	gi166156342					
Serruria aemula			gi188529251			
Serruria fasciflora			gi125858795			
Serruria longipes	gi166156340					
Serruria barbigera					gi268526664	gi268526667
Serruria scoparia					gi268526666	gi268526669
Serruria villosa					gi268526665	gi268526668
Sleumerodendron austrocaledonicum	gi193957811	gi194267347			gi188529227	gi188529232
Sorocephalus pinifolius			gi188529253			
Sorocephalus alopecurus			gi125858801			
Spatalla curvifolia			gi125858807			
Spatalla incurva			gi188529255			
Sphalmium racemosum		gi3850935	gi133930664			
Stenocarpus salignus	gi166156344	gi3850981		gi24181797	gi24181651	gi24181696
Stenocarpus sinuatus	gi166156346		gi4098561			
Stirlingia latifolia	gi166156348	gi3850913	gi133930642			
Strangea linearis			gi188529312			
Symphionema montanum	gi166156350	gi3850907	gi133930636			
Synaphea media		gi3850917				
Synaphea spinulosa	gi166156354					
Synaphea polymorpha	gi166156352					
Telopea speciosissima	gi166156356		gi4098563			
Telopea oreades			gi133930696			
Toronia toru		gi3850903	gi133930632			
Triunia montana		gi3850939				
Triunia youngiana	gi166156288		gi133930668			
Turrillia lutea	gi193957813	gi194267349	gi188529314			
Vexatorella alpina	gi166156360		gi188529257			
Virotia neurophylla	gi193957827	gi194267363				
Virotia leptophylla			gi188529316			
Xylomelum scottianum		gi3850955				
Xylomelum pyriforme			gi133930680			
Xylomelum angustifolium	gi166156362					
Platanus occidentalis	EU642711	POU86386	PTNCRBCL	AY832235	AY145358	
Nelumbo nucifera	AM396514	GQ997549	NELCPRBCOB	GQ997598	FJ626571	
Sabia swinhoei	HE651034	AF093395	FJ262616	HE651054	FJ626572	
Buxus sempervirens	AF186397	AF092110	HM849831	HE651065	AY145357	

Table S2: Fossil calibration points for the Proteaceae phylogeny. All absolute ages in million years ago (Ma) follow the geological timescale of Gradstein et al. (2004). ? indicates uncertainty in the assignment by the authors cited, Minimum age (Min. Age) refers to the upper (youngest) bound of the oldest geological stage in which the fossil has been confirmed.

Fossil taxon	**Reference for fossil taxon**	**Age (Ma) (Min for all except first line)**	**Reference phylogenetic assignment**	**Fossil placement in the extant taxon tree**	**Reference placement for dated Proteaceae phylogenetic analysis**
Tricolpate pollen	(Hughes and McDougall 1990)	125 (max age)	Eudicotyledonae (Hughes and McDougall 1990)	Eudicotyledonae (root)	(Sauquet et al. 2009)
Banksieaeidites elongatus	(Cookson 1950)	55.8	?Banksiinae (Dettmann and Jarzen 1998)	stem Banksieae	(Sauquet et al. 2009)
Cranwellipollis palisadus	((Couper 1953)) (Martin and Harris 1974)	70.6	?Franklandia (Martin 1995)	stem *Franklandia*	(Sauquet et al. 2009)
Granodiporites nebulosus	(Stover and Partridge 1973)	35.4	*Embothrium* (Martin 1995, Dettmann and Jarzen 1998)	stem *Embothrium*	(Sauquet et al. 2009)
Propylipollis crotonoides	(Dettmann and Jarzen 1996)	70.6	Grevilleoideae (Dettmann and Jarzen 1996)	crown Macadamieae	(Sauquet et al. 2009)
Triorites africaensis	(Jardiné and Magloire 1965)	93.6	Proteaceae, ?as a stem relative (Ward and Doyle 1994)	stem Proteaceae	(Sauquet et al. 2009)
Musgraveinanthus alcoensis	(Christophel 1984)	33.9	Musgraveinae (Christophel 1984)	stem Musgraveinae	(Sauquet et al. 2009)
Conchotheca rotundata	(Mueller 1873)	23	*Grevillea* (Dettmann and Clifford 2005)	stem Grevilleeae	*this study*[1]
Propylipollis ambiguus	(Stover and Partridge 1973)	70	*Telopea, Oreocallis* (Dettmann and Jarzen 1998)	stem Embothriinae	(Barker et al. 2007)
Beaupreaidites elegansiformis	(Cookson 1950)	70.6	*Beauprea* (Dettmann and Jarzen 1998, Milne 1998)	stem *Beauprea, Faurea, Protea*	(Sauquet et al. 2009)
Persoonieaephyllum	(Carpenter et al. 2010)	21.7	Persoonieae (Carpenter et al. 2010)	Persoonieae stem	*this study*[2]
Agastachys odorata	n.a. - extant species	2.6	*Agastachys odorata* (Jordan 1995)	stem *Agastachys odorata*	*this study*[3]
Orites excelsoides, O. milliganoides and O. scleromorpha	(Carpenter and Jordan 1997, Jordan et al. 1998)	28.1	*Orites* (Carpenter and Jordan 1997, Jordan et al. 1998)	*Orites* crown	*this study*[4]

Megahertzia (?amplexicaulis)	(Christophel and Greenwood 1987)	38	*Megahertzia* (Christophel and Greenwood 1987, Carpenter 1994)	stem *Megahertzia*	*this study*[5]
Propylipollis annularis	(Cookson 1950)) (Martin and Harris 1974)	70.6	?*Xylomelum* or *Lambertia* (Dettmann and Jarzen 1998) (Milne 1994, Askin and Baldoni 1998)	crown Grevilleoideae	(Sauquet et al. 2009)

[1] Dettmann and Clifford (2005) argued that *Conchotheca rotundata* is morphologically consistent with fruits of *Grevillea*. Characters shared between fossil and extant fruits are: asymmetrical except about the plane of potential dehiscence; a dorsal hinge line shorter than ventral suture; lateral attachment of seeds; and radially aligned fibres in the middle and outer pericarp. These characters were not assessed cladistically, but the authors recognised that Grevilleeae (i.e. *Grevillea*, *Hakea* and *Finschia*) fruits are distinct from those of the sister of Grevilleeae, *Buckinghamia*. In this genus the dorsal hinge line is longer than the ventral suture, and the radially directed vascular bundles of the middle and outer pericarp are branched (*vs* unbranched in *Grevillea*). Dettmann and Clifford (2005) also noted that because seed material was absent from the fossil specimens they examined, the fossil affinity was not unambiguously with *Grevillea.* Therefore, for our analysis, the fossils are placed at stem level for tribe Grevilleeae.

[2] Carpenter et al. (2010) referred foliar material from the Oligo-Miocene of New Zealand to Persoonieae based on a combination of leaf and cuticular traits found only in that clade, including the apparent synapomorphy for subfamily Persoonioideae of extremely large stomatal size (guard cells >50µm long). The features of the fossils did not allow placement in any of the currently recognised extant genera of Persoonieae (*Acidonia*, *Garnieria*, *Persoonia* and *Toronia*). Therefore, *Persoonieaephyllum* is placed at stem level for tribe Persoonieae.

[3] Jordan (1995) reported the presence of *Agastachys odorata* leaf and cuticular remains in Tasmanian Pleistocene sediments, from within the current geographical range of the species. Although there is similarly no reason to doubt this identification given the recent age of the fossils, Carpenter (2012) noted that the leaves (and cuticle) of *A. odorata* are not particularly distinctive, and so we accept the fossils as representing the genus at stem level only.

[4] Carpenter (2012) justified three early Oligocene Tasmanian fossil *Orites* species as belonging to the crown group, on the basis that each has synapomorphies for a subclade of the genus according to an unpublished (A. R. Mast & P. H. Weston) topology of species relationships. *Orites excelsoides* (described by Carpenter and Jordan 1997) is difficult to distinguish from extant *O. excelsus* in all available leaf and cuticular characters, and in particular shares a uniquely derived feature in *Orites* of having wax on the abaxial cuticle surface that obscures the positions of the stomata (Carpenter 1994). *Orites milliganoides* and *O. scleromorpha* (both described by Jordan et al., 1998) are small leaves that share marked similarities with the small, sclerophyllous leaves of extant *O. acicularis* and *O. milliganii*. The fossils are confidently assigned to the clade comprising these species because the fossil and extant species share the synapomorphy of a specific form of sclerified hypodermis, which is not known in other plants (Jordan et al. 1998, Jordan et al. 2005).

[5] Carpenter (2012) provisionally accepted Eocene fossils first reported by Christophel et al. (1987) as belonging to the monotypic *Megahertzia*. The architecture and cuticle of the fossils closely matches that of *M. amplexicaulis*, sharing the same type of lobing and teeth, prominent fine striations on both outer surfaces, relatively rare trichome bases that are associated with numerous basal epidermal cells, sinuous to buttressed anticlinal cell walls and granular inner cuticle surfaces (Carpenter, 1994). Moreover, although most fossil specimens so far examined do not have well-preserved leaf bases, these bases appear to be auriculate, a state that is apparently uniquely derived in tribe Roupaleae. We therefore include the fossils as stem *Megahertzia*.

Table S3: Proteaceae clade support and divergence times (crown-group ages) based on the Maximum Clade Credibility (MCC) tree resulting from BEAST. These are compared to mean age estimates resulting from the genus-level BEAST analysis by Sauquet et al. (2009). C = constrained node, p.p. = posterior probability, HPD = Highest posterior density, n.a. = not applicable.

Family / Subfamily	Tribe	Subtribe / genus	Con-strained or p.p.	Age (median)	Age (95% HPD)	Age (mean) Sauquet et al. 2009
Proteaceae			C	107.2	96.1 – 115.5	91.4
Persoonioideae	Persoonieae		C	45.7	21.6 – 79.1	20.8
Symphionematoideae			C	25.0	7.3 – 78.4	44.8
Proteoideae			C	85.5	74.8 – 92.2	80.8
	Conospermeae		C	43.2	26.2 – 66.2	56.5
		Conosperminae	C	26.5	15 – 52.7	36.1
	Petrophileae		C	45.3	21.7 – 67	49.2
	Proteeae		C	47.3	38.9 – 69.3	30.3
	Leucadendreae		C	69.5	41.9 – 71.5	44.5
		Leucadendrinae	C	50.3	29.3 – 58.4	28.1
		Isopogon	C	32.5	9.5 – 52.6	n.a.
		Adenanthos	C	21.2	0.6 – 34	n.a.
Grevilleoideae			C	90.1	87.2 – 109.6	80.4
	Roupaleae		C	77.8	57.3 – 93	61.8
		Roupalinae	C	64.7	35.8 – 76.6	34.9
		Lambertiinae	C	35.2	17.2 – 50	35
		Heliciinae	C	26.8	1.1 – 29.7	5.5
	Banksieae		C	64.6	50.4 – 83.7	50.3
		Musgraveinae	C	58.3	35.3 – 74	9.12
		Banksiinae	C	47.8	38.6 – 72.1	21.4
	Embothrieae		C	76.5	70 – 93.1	66.6
		Lomatia	C	22.9	7.1 – 31.5	n.a.
		Embothriinae	C	58.9	35.4 – 70.2	41.1
		Stenocarpinae	0.4	21.3	3.2 – 51.8	36.5
		Hakeinae	C	62.6	52 – 81	45.4
	Macadamieae		C	78.7	70.6 – 94.3	72.7
		Macadamiinae	C	56.6	36.7 – 78	38.1
		Malagasiinae	C	27.9	0.5 – 35	13.7
		Virotiinae	1	26.8	7.6 – 47.1	33.2
		Gevuininae	C	65.6	32.3 – 76.5	37.4

Table S4: OUwie model selection based on AICc scores for each model for each variable. Models are described in Table 2. In **bold** the selected model with the lowest AICc score. w = AIC weight, n.a. = not applicable.

Variable	BM	OU	OU_2	BM_σ	$OU_{2\sigma}$	$OU_{2\alpha}$	$OU_{2\sigma\alpha}$	w
Shape PC1	229.4	-94.4	-94.5	155.5	**-104.2**	n.a.	n.a.	0.98
Shape PC2	59.8	-193	-191.6	1.2	**-201.2**	n.a.	n.a.	0.98
Leaves PC1	1079	918.9	881.9	990.9	**831.3**	n.a.	n.a.	1
Leaves PC2	1145.5	816.8	816	1082.9	**804.7**	n.a.	n.a.	0.99
Log (blade area)	843.7	770.4	725.4	797.1	**699.4**	725.7	701.5	0.74
Log (effective leaf area)	1148.1	951.5	**897.2**	1064.8	n.a.	n.a.	n.a.	1
Log (SLA)	533.8	338.7	305.3	472.5	**289.0**	n.a.	n.a.	1
Log (blade perimeter)	718.1	551.2	532.6	664.2	**514.4**	n.a.	n.a.	1
Circularity	177.5	-59.5	-59.2	136.2	**-67.8**	n.a.	n.a.	0.97
Log (Feret-diameter)	860.5	611.9	597.5	796.1	**575.5**	n.a.	n.a.	1
Log (Complexity)	570.5	324	316.6	492.8	**283.8**	n.a.	n.a.	1
Log (Dissection)	277.1	181.3	**171.8**	246.9	173.4	172.8	175.1	0.44
Log (Feret-diameter ratio)	850	500.1	498.6	795.5	**494.8**	n.a.	n.a.	0.82
Log (Perimeter / area)	693.6	594.4	548.3	624.5	505.8	550.2	**503.9**	0.72
Log (Teeth / perimeter)	326.5	218.3	**217.9**	313.9	220	219	n.a.	0.37
Log (Teeth / blade area)	351.0	278.4	**259.9**	341.6	261.9	262.1	264.2	0.56
Climate PC1	1015	797.2	625	961.8	**621.9**	n.a.	n.a.	0.83
Climate PC2	910	685.1	**656.7**	876.1	658.5	n.a.	n.a.	0.71
MAT	1896.5	1656.8	**1567.9**	1866.3	1569.3	n.a.	n.a.	0.67
MinTColdMonth	1160	1109.5	1042.2	1160.9	**1031.6**	1043.1	1033.3	0.7
MaxTempWarmMonth	1895.8	1685.5	1686.1	1867.5	1681.7	1671.3	**1670.8**	0.55
√ (PrecipWettestQ)	1644.8	1348.1	1183.6	1594.8	**1169.9**	n.a.	n.a.	1
√ (PrecipDriestQ)	1189.6	1020.2	**974.1**	1179.6	976	n.a.	n.a.	0.73
√ (PrecipColdestQ)	1629.1	**1085**	1086.9	1568.7	1088	n.a.	n.a.	0.63
√ (PrecipWarmestQ)	1386.3	1350.1	1256.8	1383.9	1258.3	**1254.4**	1256.5	0.56
√ (MAP)	1369.2	1334.3	1226.6	1362.1	1226.9	1227.3	**1225.1**	0.46

Figure S1
Constrained Proteaceae input topology for the BEAST analysis.

Shape PC1
Climate PC1
Shape PC1
Climate PC2
Shape PC2
Climate PC1
Shape PC2
Climate PC2
Leaves PC1
Climate PC1
Leaves PC1
Climate PC2
Leaves PC2
Climate PC1
Leaves PC2
Climate PC2
log (blade area)
Climate PC1
log (blade area)
Climate PC2
log (effective leaf area)
Climate PC1
log (effective leaf area)
Climate PC2
log (SLA)
Climate PC1
log (SLA)
Climate PC2
log (blade perimeter)
Climate PC1
log (blade perimeter)
Climate PC2

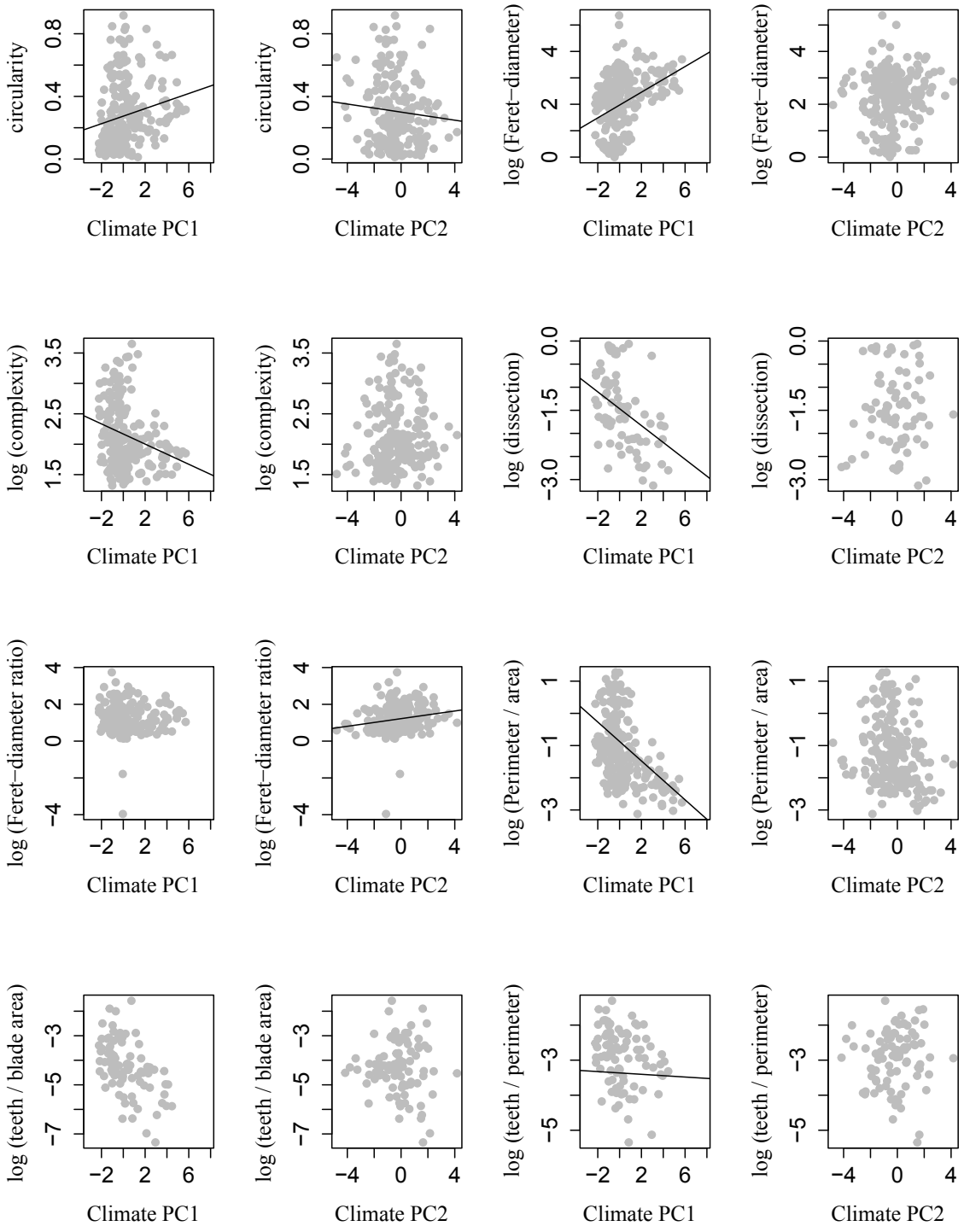

Figure S2
Phylogenetic generalized least squares regression of traits to climate principal components (PCs) in Proteaceae, based on 216 species. If the effect of climate PCs on variation in traits was detected to be significant with PGLS regression, the regression line (black) was plotted. Dots represent species.

CHAPTER VI: ON THE COMPLEXITY OF TRIGGERING EVOLUTIONARY RADIATIONS

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Author contributions:

YBK, REO, YX, OS and HPL designed the research; YBK collected Poales trait data and provided the phylogeny; OS collected Ericaceae trait data and provided the phylogeny; YX and REO collected Fagales trait data and provided the phylogeny; YBK and OS performed BAMM diversification rate analyses; REO developed the analytical pipeline, performed BiSSE diversification rate analyses, ancestral state reconstructions and time-sequence analyses and plots (to classify backgrounds, triggers, modulators); REO made all the figures for the manuscript; YBK wrote most parts of the manuscript; REO wrote most parts of materials and methods and result; HPL gave major comments on the manuscript, YX and OS gave minor comments on the manuscript.

Summary

- Recent developments in phylogenetic methods have made it possible to reconstruct evolutionary radiations from extant taxa, but identifying the triggers of radiations is still problematic. Here, we propose a conceptual framework to explore the role of variables that may impact radiations. We classify the variables into extrinsic conditions versus intrinsic traits, whether they provide background conditions, trigger the radiation, or modulate the radiation.
- We used three clades representing angiosperm phylogenetic and structural diversity (Ericaceae, Fagales and Poales) as test groups. We located radiation events, selected variables potentially associated with diversification, and inferred the temporal sequences of evolution.
- We found thirteen shifts in diversification regimes in the three clades. We classified the associated variables, and determined whether they originated before the relevant radiation (backgrounds), simultaneously with the radiations (triggers), or evolved later (modulators).
- By applying this conceptual framework, we establish that radiations require both extrinsic conditions and intrinsic traits, but that the sequence of these is not important. We also show that diversification drivers can be detected by being more variable within a radiation than conserved traits that only allow occupation of a new habitat. This framework facilitates exploration of the causative factors of evolutionary radiations.

Keywords:

adaptive zone, angiosperms, diversification drivers, exaptations, key innovations, radiation, diversification rate shifts, triggers.

Introduction

Evolutionary radiations usually imply two processes: multiplication of species (or increased taxonomic diversity) and increased phenotypic disparity (Givnish 1997, Schluter 2000, Losos 2009, Glor 2010, Losos and Mahler 2010). In this paper we use the term 'radiation' to refer to the proliferation of species only (i.e. 'explosive speciation' *sensu* Givnish (2010)), rather than the accelerated diversification of ecological roles *sensu* Givnish (1997). We therefore understand radiation as a significant increase in the taxonomic diversification rate. Clades that have at least an order of magnitude more species than their sister clades are regarded as having undergone radiations (Sanderson and Donoghue 1994).

In angiosperms, birds and mammals, and possibly also other clades, much of the current species richness is the result of radiation events (O'Leary et al. 2013, Moen and Morlon 2014, Zanne et al. 2014). Understanding the causes and constraints of radiations is an important step in the study of the evolution of diversity. Radiations were initially explored by palaeontologists, who demonstrated high diversification rates after mass extinctions, followed by a slowdown in the diversification rate (Stanley 1979). Molecular phylogenies and recent developments in phylogeny-based macroevolutionary inference have made it possible to infer radiations from extant taxa with greater precision and objectivity than before. This has stimulated much research to establish a critical protocol for estimating and locating radiation events and their correlates, which can be understood to be potential triggers of these radiations. This led to the development of a tool box for identifying rate shifts and linking these to potential triggers (Pybus and Harvey 2000, Maddison et al. 2007, Alfaro et al. 2009, FitzJohn et al. 2009, FitzJohn 2010, Stadler 2011, FitzJohn 2012, Rabosky 2014).

Simpson (1953) developed a model for radiations that involves 'more or less simultaneous divergence of multiple lineages […]'. He suggested that radiation was a consequence of entering a new adaptive zone. The adaptive zone can be understood to be a geographical area or a habitat, in which the radiating lineage could expand taxonomically, structurally and/or ecologically. Typical adaptive zones could be isolated islands or unusual habitats such as epiphytic or on oligotrophic soils for plants. In order to enter an adaptive zone, three conditions need to be satisfied. Firstly, the lineage needs physical access to this zone, and this is often the result of dispersal. Secondly, access to a new adaptive zone could be facilitated by the evolution of appropriate traits, that is, features that allow the organism to occupy novel environments and to interact with existing environments in a novel way, which may facilitate later ecological divergence. Finally, Simpson (1953) argued that the zone should either be empty, or that the occupants are competitively inferior. These two ideas of ecological opportunity (an adaptive zone *sensu* Simpson (1953)), and evolutionary change needed to occupy this zone, and the interplay between them, have dominated recent thinking about and explanations of radiations (Moore and Donoghue 2007, Drummond et al. 2012b).

Due to lack of complete fossil records for the vast majority of organisms, interpreting radiations using fossils alone has many limitations and constitutes a difficult task (Kidwell and Holland 2002, Adrain and Westrop 2003). At least since the work on the radiation of phytophagous insects by Mitter et al. (1988) , which was based on phylogenies derived from extant organisms, radiation has been increasingly interpreted as a rapid evolution of species richness. The adaptive zone was now referred to as the 'ecological opportunity', and the evolutionary change that allowed access to the ecological opportunity as a 'key innovation'. However, key innovations can also initiate radiations by decreasing the probability of extinction via increased individual fitness, or by favouring reproductive and/or ecological specialization (Heard and Hauser 1995). Simplistically, if the key innovation is only understood as enabling access to the ecological opportunity and accelerated generation of diversity constitutes a radiation, then, Glor (2010) and Losos (2010) argued that an additional attribute is needed: a driver of diversification. This driver could be intrinsic, such as a set of traits that allow for faster speciation, or extrinsic, such as the occupation of a complex habitat. Both these conditions could affect speciation rate by accelerating reproductive isolation. Thus the 'Simpsonian' model, with appropriate modifications, is still useful in the molecular era.

Early phylogenetic work contrasted sister clades and sought correlates of significant differences in the richness of the clades, and these traits were then interpreted to be key innovations (Mitter et al. 1988, Hodges and Arnold 1995a). With more detailed phylogenies becoming available, and especially with the generation of time-calibrated branch lengths, much more detailed analyses became possible, using algorithms such as the binary state speciation and extinction algorithm (BiSSE) (Maddison et al. 2007). Case studies soon revealed that the situation can be quite complex, and that the apparent key innovations may evolve before the ecological opportunities and the initiation of the radiations, or vice versa. The notothenioid fishes, for example, evolved antifreeze glycoproteins 42 – 22 Ma, some 10 My before the Antarctic waters became very cold, and the species radiation was initiated (Near et al. 2012). The BiSSE algorithm can be used to detect evidence for trait-dependent diversification and differential diversification rates, but these do not necessarily coincide with diversification rate shifts or radiations. A radiation constitutes a whole clade with an, on average, accelerated diversification rate, while changes in diversification rate could be scattered across a phylogeny without any specific radiating clade. There seems to be no general protocol for locating triggers of radiations.

The variables used to investigate a correlation with diversification rates are generally simplifications of the much more complex biological and environmental processes affecting diversification rate heterogeneity in a group of organisms. It may not always be easy to establish which variables summarize which complex traits in the biology of the species, yet it is the full

biological syndrome that facilitates the radiation (Verdú and Pausas 2013). C_4 photosynthesis is an example of such a complex trait because it is coupled with physiological features, specialized leaf anatomy and organelle structure and distribution (Laetsch 1974). C_4 plants fix CO_2 via the Calvin cycle leading to a four-carbon compound instead of the three-carbon compound produced by C_3 plants (Sage 2004), and they also display a unique 'Kranz' anatomy (Hamberlandt 1904). This whole complex could be quantified by a single variable, such as the $\delta^{12}C$:$\delta^{13}C$ isotopic ratio. To complicate matters, it has been shown that foliar anatomy (i.e. proportion of bundle sheath tissue) preceded and facilitated the evolution of C_4 photosynthesis in grasses (Christin et al. 2013). A similar nested arrangement of characteristics is also found in the complex orchid flowers. Here, the evolution of the floral structure involved, more or less in sequence, the evolution of an inferior ovary, zygomorphy in the perianth, reduction in the number of stamens to one, fusion of pollen into pollinia, fusing of the stamen and style into a gynostemium and development of a viscidium (Rudall and Bateman 2002). The last trait which makes the syndrome fully functional can therefore be regarded as the key innovation. The traits do not need to be so fully integrated, but could all be responding to the same environmental conditions. The leaf economic spectrum describes the balance of investment in leaves, ranging from cheap, short-lived, high-return leaves to expensive, long-lived, low-return leaves (Wright et al. 2004, Cornwell et al. 2014), and these are linked to the productivity of the environment. Specific leaf area (SLA), the ratio between leaf area and leaf weight (mm^2/mg), is one of the components of the spectrum, and sometimes used as a proxy for other traits, such as leaf nitrogen, photosynthetic capacity and leaf longevity (Onstein et al. 2014). However, unlike the C_4 photosynthetic pathway, or the orchid flowers, the traits that make up the leaf economic spectrum are not necessarily tightly associated. A further complication, which has recently been disentangled, is the occurrence of cryptic precursor traits, postulated to be necessary for the key innovations to evolve (Marazzi et al. 2012, Werner et al. 2014). For example, Werner et al. (2014), in an angiosperm-wide analysis, detected an over 100 My old, single and cryptic evolutionary innovation of symbiotic N_2-fixation, followed by multiple gains (e.g. in the Cucurbitales, Fabaceae, Fagales and Rosales) and losses of the symbiosis.

In order to better understand the likely triggers of radiations, we developed an explicit conceptual framework and classification of variables as they relate to radiations. This framework scores variables for three attributes: 'types', 'timings' that can be used to characterize the temporal sequence of variable shifts in relation to the initiation of radiation, and 'roles' that distinguish different functions of variables during diversification.

The first attribute (the 'type') distinguishes between variables that represent extrinsic conditions versus intrinsic traits. Extrinsic conditions refer to all conditions outside the organisms, such as physical space, climate, other organisms, and any form of habitat not yet occupied or not effectively occupied. These describe Simpson's adaptive zone. Intrinsic traits evolve with the organism and thus can be physiological or morphological 'functional traits' which affect the fitness of the organism through their effects on growth, survival and reproduction (Violle et al. 2007).

The second attribute (the 'timing') divides these variables (both extrinsic and intrinsic) into three groups based on their temporal relationship to the start of the radiation. Background variables ('backgrounds' hereafter) are established before the radiation starts and are necessary to create the conditions in which a radiation can start. If only considering extrinsic variables, backgrounds could be regarded as 'key landscapes' *sensu* Givnish (1997). If they are intrinsic, Lieberman (2012) refers to these variables as exaptations, a term adopted from Gould and Vrba (1982). The second group consists of the trigger variables ('triggers' hereafter) of the radiations, which have to be established contemporaneously with the start of the radiation. The third group consists of modulator variables ('modulators' hereafter) of the radiation. These variables become established after the start of the radiation. These could function to fine-tune the radiation to its environmental condition, or could

impart an additional impulse to the radiation. Importantly, modulators could therefore also be triggers for nested radiations.

The third attribute (the 'role') divides the variables according to their presumed functions as diversification drivers. These include, firstly, those variables that primarily facilitate survival of the lineage. These variables should therefore be phylogenetically conserved and invariable during the radiation. We refer to them as 'simple' variables. The second group includes those variables that stimulate and/or maintain diversification (Glor 2010, Losos 2010). Theory predicts that these should be phylogenetically labile in the radiation and thereby provide numerous ways in which closely related species can coexist. We refer to these as 'polymorphic' variables. Polymorphic variables may potentially stimulate speciation rates by partitioning the environment or niche of the organism, such as key innovation (Hodges and Arnold 1995a) or traits which cause ecological divergence (Carlson et al. 2011) or, in case of extrinsic conditions, environmental heterogeneity providing multiple niches for ecological adaptation (Rosenzweig 1995, Hughes and Eastwood 2006, Antonelli and Sanmartin 2011).

Here, we test whether this paradigm can be used to explore evolutionary radiations, using three clades selected from across the phylogenetic and structural diversity of angiosperms. In order to set up a framework for classifying and identifying the variables associated with radiations, we ask whether (a) as suggested by Simpson (1953), extrinsic and intrinsic variables are most common as backgrounds and triggers, respectively; (b) modulators and backgrounds are more likely to be polymorphic and simple traits, respectively; (c) this framework could be used in radiation studies for selecting and filtering variables that are linked to radiations; and (d) the list of variables is adequate to account for the observed radiations.

Materials and Methods

Protocol and Clades

To explore evolutionary radiations, we devised the following protocol. We firstly selected clades (Table 1) which may have radiations, and then located the radiation events without any *a priori* assumption of the topological position of these radiations. Our focus was on discrete radiations (i.e. radiating clades), not on accelerations in the diversification rates (i.e. radiating lineages), which can be diffuse across a phylogeny. Secondly, we selected the extrinsic and intrinsic variables which could be correlated to the radiations (i.e. backgrounds, triggers or modulators). Thirdly, we tested whether these variables had a significant effect on net diversification rates (speciation rate minus extinction rate) through their effect on speciation and extinction dynamics. We filtered out those variables which did not have a significant effect on the net diversification rate, and which are therefore not likely to be potential causes of radiations. Then, we established the temporal sequence of all the remaining variables in relation to the initiation of the radiation to classify the variables into backgrounds, triggers or modulators, and to evaluate their role within the radiation as simple (conserved) or polymorphic (labile) variables.

Locating radiations

We used published dated phylogenetic hypotheses for Ericaceae (Schwery et al. 2014), Fagales (Xing et al. 2014) and Poales (Bouchenak-Khelladi et al. 2014) (Table 1). For all three clades, maximum clade credibility (MCC) trees were generated using BEAST v1.7 and v1.7.5 (Drummond et al. 2006, Drummond and Rambaut 2007, Drummond et al. 2012a). These three phylogenetic trees represented the widest sampling for each clade to date (Ericaceae: 450 out of 4,426 spp, Fagales: 515 out of 1,317 spp, and Poales: 545 out of 20,000 spp), and reflect the taxonomical, geographical, ecological and morphological diversity of the relevant clade.

In order to detect significant changes in the diversification dynamics (speciation and extinction rates), we analyzed the MCC tree of each of the three clades with BAMM 1.0 (Rabosky 2014), after excluding the outgroups. BAMM uses reversible-jump Markov chain Monte Carlo (MCMC) to select between models that vary in the number of diversification regimes, thus accounting for rate variation through time and among lineages. We assigned sampling fractions at family and subfamily (Poales), tribal (Ericaceae) and generic (Fagales) levels to include the effect of species not sampled in the phylogeny on diversification heterogeneity. We ran two MCMCs for 100,000,000 generations with a sampling frequency of 1,000 for each clade. We checked for convergence for each run by plotting the log-likelihood trace of the MCMC output file and checked that the effective sample sizes of the runs exceeded 200. Using the 'BAMMtools' package (Rabosky 2014) in R, we identified the 95% credible set of distinct shift configurations and the overall best set of rate shifts given the data. These shifts in diversification dynamics are referred to as initiation of radiations, except where there is a slow down in net diversification rate. It is possible that a more detailed sampling, especially in the Poales where the sampling fraction was estimated down to subfamily level, might have led to more detected changes in the diversification dynamics.

Table 1
Clades used in this analysis, the sampling for the phylogenetic analysis, DNA loci used to infer phylogeny and number of diversification dynamics shifts detected.

Clades	Higher group	Species	Species sampled	DNA loci	References	Diversification dynamics shifts
Ericaceae	Ericales	4'426	450	*rbc*L, *mat*K	Schwery *et al.* (2015)	6
Fagales	Rosids	1'317	515	*rbc*L, *mat*K, *trnL-F, ITS,* Crabs Claw	Xing *et al.* (2014)	4
Poales	Monocots	20'000	545	*rbc*L, *ndhF*	Bouchenak-Khelladi *et al.* (2014)	5

Candidate variables affecting radiation and their type
Ideally all variables that could influence diversification should be investigated. Here, however, we selected those variables for which we had some prior indication that they could be of importance. For the intrinsic variables, we used physiological arguments of improved performance given the ecological opportunities, whilst extrinsic variables describe the habitats where the relevant clade is most common in. These variables were assigned a 'type' by dividing them into extrinsic conditions and intrinsic traits. We did not explicitly group the variables into syndromes (as in Givnish et al. 2014), as covariation in the variables would be evident when mapped on the phylogeny.

Variable states were assembled in a species × variable matrix and scored, where possible, for all species present in the phylogenetic trees of Ericaceae, Fagales and Poales. As these variables are often phylogenetically conserved within a genus, scoring followed a phylogenetic top-down approach, in which all species in a genus would be assigned a similar state of a variable if our source of information confirmed this. In some cases (e.g. evergreen/deciduous in *Quercus*) the trait was variable within genera, in which case we scored it for each species. In other cases (e.g. specific leaf area (SLA) data for Ericaceae), there are missing data in our matrix. For all variables states, we scored presence-only (Hardy and Linder 2005) because states of a variable may not always be exclusive (e.g. a species can have a shrubby as well as a tree-like growth form). This resulted in a binary (presence/absence)

data matrix for each state of a variable (see Supporting Information Methods S1).

Filtering variables affecting radiations

BiSSE (Maddison et al., 2007), implemented in diversitree 0.4-5 (FitzJohn et al., 2009, 2012), was used to test the hypothesis that presence/absence of a variable was correlated with different diversification rates on the MCC tree. BiSSE employs maximum likelihood optimization to estimate absolute rates of asymmetric character change (q), speciation (λ), and/or extinction (μ) by maximizing the likelihood of these parameters for a given topology with branch lengths (Maddison et al., 2007). We compared the fit of seven diversification models (see Supporting Information Methods S1) in which speciation and/or extinction and/or transition rates were constrained to have similar rates for presence/absence of the variable with a likelihood-ratio test and AIC. The model with the least number of parameters which did not perform significantly worse than the full six-parameter model (χ^2 distribution $p < 0.05$, $\Delta AIC > 2$) was chosen as the best model for each variable. This model was used in a MCMC in BiSSE to estimate the posterior density of the parameters (λ, μ and q) for each state (presence/absence). We ran 10,000 MCMC iterations for every clade and each variable. By examining the 95% credible intervals of the posterior samples for each parameter, we inferred posterior Bayesian support for a difference in diversification rates between states (FitzJohn et al., 2009). If the presence of a variable was associated with increased rates of diversification, it was retained in the set of backgrounds, triggers and modulators.

Separating backgrounds, triggers and modulators

In order to determine the temporal order of the variables in relation to the shifts in diversification dynamics identified by BAMM, we optimized the retained variables which may be causing radiations on the MCC tree (without outgroups) using 'asr.marginal' implemented in the R package diversitree. Ancestral reconstructions were made using the estimated parameters (transition rates among character states) of the best model found in BiSSE, which automatically corrects for the influence of diversification rate differences when inferring ancestral states. We obtained the marginal probability of each state (presence/absence) for each variable at each node. In addition, we obtained the 95% highest posterior density (HPD) of the estimated node age from BEAST, based on a minimum of 10,000 post burn-in trees.

We divided each study-clade (i.e. Ericaceae, Fagales and Poales) into separate partial trees for each of the radiations. These partial trees included the nodes which led up to the respective radiation (i.e. the nodes from the root of the tree towards the radiations), and those included in the radiation (Fig. 1). Nodes which did not play a role in the evolutionary pathway leading to the radiation (for example in sister clades of the radiating group) were excluded. The change in probability of presence of a variable was assessed over time by plotting this probability against the median age and the 95% HPD of each node (Fig. 1). This allowed us to assign the 'timing' of each variable. Variables which exceeded a probability of 0.75 prior to the rate shift, at a node which did not overlap with the 95% HPD of the node where the radiation was initiated, were defined to be backgrounds (Fig. 1a). Variables which exceeded a probability of 0.75 at a node which overlapped with the initiation of the radiation were interpreted to be potential triggers (Fig.1b). Variables which exceeded a probability of 0.75 after the rate shift (i.e. at a node which did not overlap in time with the initiation of the radiation) were interpreted to be modulators (Fig. 1c). Backgrounds, triggers and modulators were then classified into their 'role': 'simple' if they were conserved within the radiating clade (i.e. more than 95% of the nodes having a probability of ≥ 0.75 throughout the radiation), or 'polymorphic' if they were labile within the radiating clade (i.e. probabilities of ≥ 0.75 as well as < 0.75) (Fig. 1d).

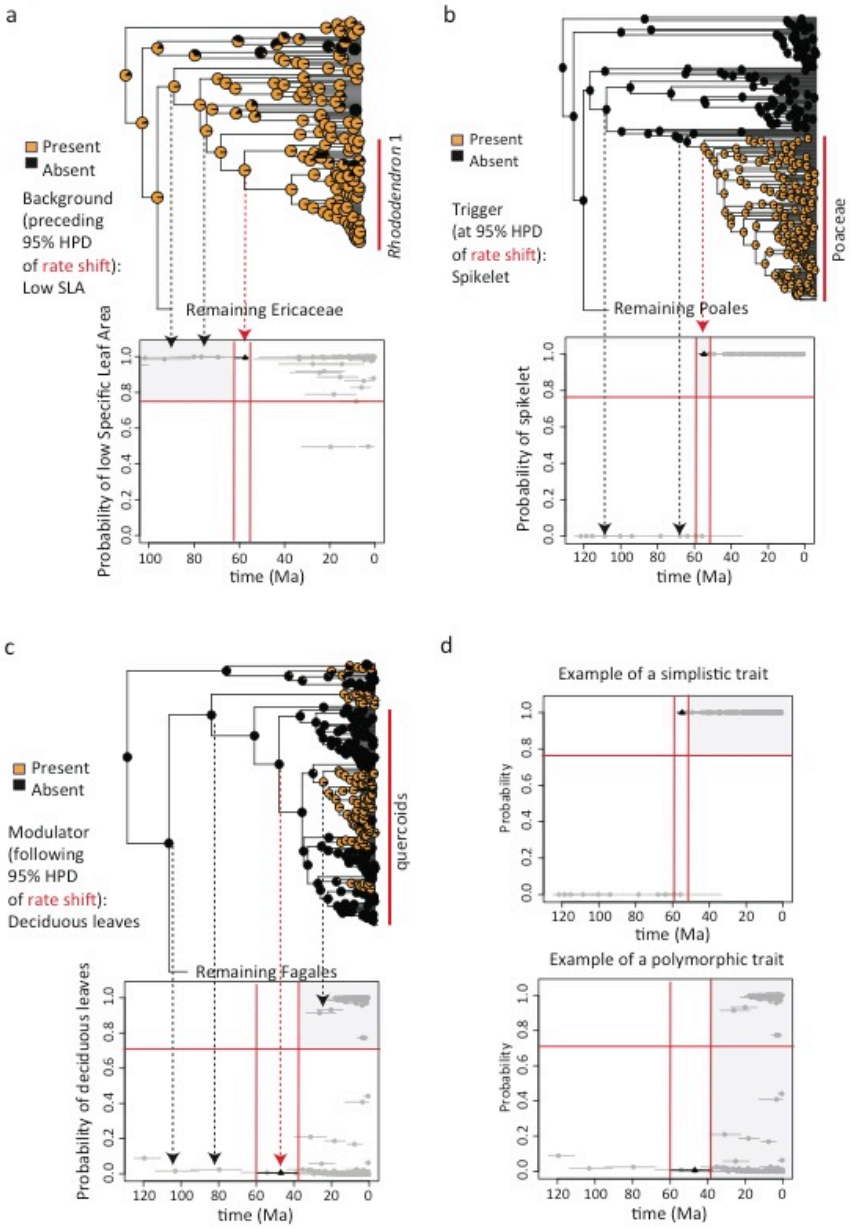

Figure 1

Diagram explaining how variables have been classified as background (panel **a**), trigger (panel **b**) and modulator (panel **c**), and simple (conserved) or polymorphic (labile) (panel **d**). All nodes leading and belonging to the radiating clade are plotted onto a graph with ancestral state probability of a variable, as reconstructed under the BiSSE model, on the y-axis and time in Ma (million years ago) on the x-axis. Dashed arrows illustrate the connection between nodes in the tree and their position on the graph. Black and grey horizontal bars of the nodes represent the 95% HPD of the node age. Black triangles represent the node at which a shift in diversification regime was found. Vertical and horizontal red lines on the graphs indicate threshold (0.75) for significant ancestral reconstructions (horizontal), and the time interval (vertical) in which significantly optimized nodes are considered backgrounds (i.e. when significantly optimized ancestral nodes are preceding the rate shift; panel **a**), triggers (i.e. when the first significantly optimized ancestral node overlap the 95% HPD of the rate shift; panel **b**), or modulators (i.e. when optimized nodes occur after the rate shift; panel **c**).

Results

Radiations

In the three clades, our phylogenetic results were largely congruent with those previously published (e.g. Ericaceae (Kron et al. 2002, McGuire and Kron 2005, Bush et al. 2009, Gillespie and Kron 2010), Fagales (Sauquet et al. 2012, Zhang et al. 2013) and Poales (Givnish et al. 2010, Briggs 2011, Bouchenak-Khelladi et al. 2014, Briggs et al. 2014)). Time-calibrated phylogenies are available in Dryad Digital Repository (http: www.datadryad.org, DOIF: 10.5061/dryad.9pg3r).

We find six radiations in Ericaceae, three in Fagales and one slowdown, and four in Poales with one slowdown (Figs 2-4).

Filtering variables affecting radiations

We found eight out of 20 intrinsic and four out of seven extrinsic variables significantly positively associated with diversification rate changes by BiSSE (Tables 2-4; see Supporting Information Fig. S1). In two clades some variables had no significant effect on net diversification rates, such as deciduous/evergreen leaves, epiphytism and tree growth form in Ericaceae (Table 2) and open tropical biomes, scale leaves and serotiny in Fagales (Table 3). Seven variables had a significant negative effect on the diversification rate; since our coding is presence only, we ignored these (Tables 2-4).

Temporal classification

For each radiation, the retained variables were separated into backgrounds, triggers, and modulators (see Supporting Information Fig. S2). Background variables were found to be either only extrinsic (two radiations), only intrinsic (five radiations), or both extrinsic and intrinsic (two radiations). Triggers were found to be only extrinsic (two radiations), only intrinsic (two radiations), or both (three radiations). Finally, modulators were found to be only extrinsic (one radiation) or only intrinsic (four radiations) (Table 5). Backgrounds were found in all radiations, except in Betulaceae, quercoids, Poaceae and Bromeliaceae (Tables 2, 4). No triggers were found for six radiations: the Cyperoideae in Poales, *Allocasuarina* in Fagales, and *Erica, Gaultheria and Rhododendron* 1 and 2*,* in Ericaceae (Tables 2-4). No modulators were found for eight radiations: Cypereae and Bromeliaceae in Poales, *Allocasuarina* in Fagales, and *Gaultheria*, *Rhododendron* 2, *Erica*, the Richeeae and Vaccinieae in Ericaceae (Tables 2-4). We did not find support for the hypotheses that backgrounds are more likely to be extrinsic variables, and triggers more likely intrinsic variables, as the frequency of the timing (background, triggers or modulators) was not significantly different between types (intrinsic or extrinsic) (χ^2 test (Preacher 2001); χ^2=1.309; P = 0.520).

Table 2

Traits, their classification, performance in BiSSE, and temporal classification relative to the diversification dynamics shifts in Ericaceae.

	Variable	Type	BiSSE	SHIFT						Ancestral
				Richeeae	*Erica*	*Rhododendron 1*	*Rhododendron 2*	*Gaultheria*	Vaccinieae	
HABITAT	Mountain	Extrinsic	↑*	trigger P	x	post P	pre P	pre P	trigger P	ambiguous
LEAF	Low SLA	Intrinsic	↑*	pre S	pre S	pre P	pre S	pre S	pre P	low SLA
	Deciduous leaves	Intrinsic	NS	x	x	post P	x	x	post P	non-deciduous
	Evergreen leaves	Intrinsic	NS	pre S	pre S	pre P	pre S	pre S	pre P	evergreen
GROWTH FORM	Shrub	Intrinsic	↑*	pre S	pre P	pre P	pre S	pre S	pre P	shrub
	Herb	Intrinsic	↓*	x	x	x	x	x	x	non-herb
	Tree	Intrinsic	NS	x	post P	post P	x	x	x	non-tree
	Epiphyte	Intrinsic	NS	x	x	x	x	x	trigger P	non-epiphyte

'↑' and '↓' represent positive and negative association with diversification rates (respectively) from the BiSSE analyses. '*' indicates statistical significance. NS indicates non significant association for the trait. 'pre', 'trigger' and 'post' refer to the temporal relationship of the trait to the radiation: background, trigger and modulator, respectively (cf. Introduction). 'P' and 'S' indicate the function of the trait to the radiation: polymorphic and simple, respectively (cf. Introduction). 'x' indicates that the trait cannot be classified relative to the model shift (i.e. probability below 0.75 for the ancestral reconstructions). Shaded rows indicate the variables selected that are associated with radiations.

Table 3

Traits, their classification, performance in BiSSE, and temporal classification relative to the diversification dynamics shifts in Fagales.

	Variable	Type	BiSSE	SHIFT				Ancestral
				Allocasuarina spp	Betulaceae spp	*Nothofagus* ¶	quercoids	
HABITAT	MTE	Extrinsic	↑*	X	x	n/a	trigger P	ambiguous
	TEF	Extrinsic	↓*	X	x	n/a	pre P	TEF
	TDF	Extrinsic	↓*	X	pre S	n/a	post P	TDF
	Tropics (open)	Extrinsic	NS	pre S	x	n/a	post P	non-tropics
WOOD POROSITY	Ring porosity	Intrinsic	↑*	X	x	n/a	trigger P	non-ring
	Semi-ring porosity	Intrinsic	↑*	pre S	post P	n/a	trigger P	non-semi-ring porous
	Diffuse	Intrinsic	↓*	pre S	pre S	n/a	pre P	diffuse
DISPERSAL	Biotic dispersal	Intrinsic	↑*	X	post P	n/a	trigger S	non-biotic
	Abiotic dispersal	Intrinsic	↓*	pre S	pre P	n/a	x	abiotic
	Passive dispersal	Intrinsic	↓*	X	x	n/a	x	passive
LEAF	Deciduous leaves	Intrinsic	↑*	X	trigger S	n/a	post P	non-deciduous
	Evergreen leaves	Intrinsic	↓*	pre S	x	n/a	pre P	evergreen
	Scale leaves	Intrinsic	NS	pre S	x	n/a	x	no scale leaves
	Serotiny	Intrinsic	NS	pre S	x	n/a	x	non-serotinous
OTHER	N_2 fixation	Intrinsic	↓	X	post P	n/a	x	non-N_2 fixation

'MTE': Mediterranean-type ecosystem, 'TEF': Tropical evergreen forest, 'TDF': Temperate deciduous forest and 'N_2 fixation': Nitrogen fixation. '↑' and '↓' represent positive and negative association with diversification rates (respectively) from the BiSSE analyses. '*' indicates statistical significance. NS indicates non significant association for the trait. 'pre', 'trigger' and 'post' refer to the temporal relationship of the trait to the radiation: background, trigger and modulator, respectively (cf. Introduction). 'P' and 'S' indicate the function of the trait to the radiation: polymorphic and simple, respectively (cf. Introduction). 'x' indicates that the trait cannot be classified relative to the model shift (i.e. probability below 0.75 for the ancestral reconstructions). '¶' shows that the rate shift found in *Nothofagus* indicates a slow down. n/a indicate that variables were not considered. Shaded rows indicate the variables selected that are associated with radiations.

Table 4

Traits, their classification, performance in BiSSE, and temporal classification relative to the diversification dynamics shifts in Poales.

	Variable	Type	BiSSE	SHIFT					Ancestral
				Poaceae	EDL ¶	Cyperoideae	Cypereae	Bromeliaceae	
HABITAT	Open	Extrinsic	↑*	trigger P	n/a	pre S	pre S	trigger P	non open
	Dry	Extrinsic	↑*	trigger P	n/a	pre P	pre S	trigger P	non dry
PHOTOSYNTHESIS	CCM	Intrinsic	↑*	post P	n/a	post P	trigger S	trigger P	non CCM
OTHER	Spikelet	Intrinsic	↑*	trigger S	n/a	x	x	x	non-spikelet

'CCM': CO_2-concentrating mechanisms (i.e. C_3 vs. C_4 and CAM). '↑' and '↓' represent positive and negative association with diversification rates (respectively) from the BiSSE analyses. '*' indicates statistical significance. NS indicates non significant association for the trait. 'pre', 'trigger' and 'post' refer to the temporal relationship of the trait to the radiation: background, trigger and modulator, respectively (cf. Introduction). 'P' and 'S' indicate the function of the trait to the radiation: polymorphic and simple, respectively (cf. Introduction). 'x' indicates that the trait cannot be classified relative to the model shift (i.e. probability below 0.75 for the ancestral reconstructions). '¶' shows that the rate shift found in EDL (Early Diverging Lineages of the graminids clade: Ecdeiocoleaceae, Flagellariaceae, Joinvilleaceae, and the early-diverging lineages of Poaceae) indicates a slow down. n/a indicate that variables were not considered. Shaded rows indicate the variables selected that are associated with radiations.

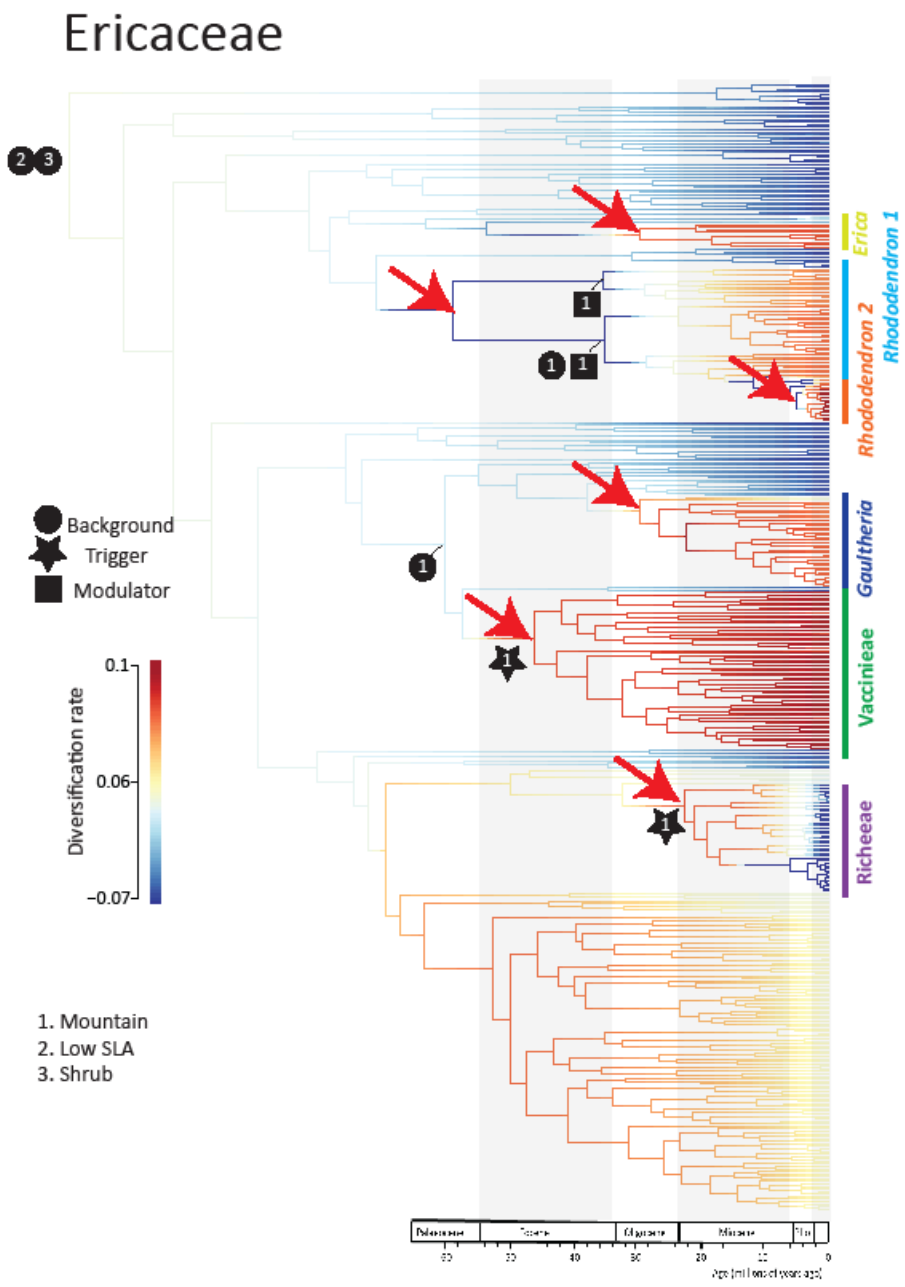

Figure 2

MCC tree from the BEAST analysis for Ericaceae. Diversification rates are shown along each branch of the phylogeny. Each color section of a branch represents the mean of the posterior density of net diversification rate. The red (speed up) arrows indicate where shifts in diversification regime occur. Each number represents one of the variables tested. We found six radiations, which correspond to the temperate Afro-European genus *Erica* (~860 spp), Australasian Richeeae (Styphelioideae) (~70 spp), *Rhododendron* 1 (~860 spp) and a nested radiation of some Himalayan members of *Rhododendron* 2 (~150 spp), and two South America – Asian clades: *Gaultheria* (~130 spp) and the tribe Vaccinieae (~1,564 spp).

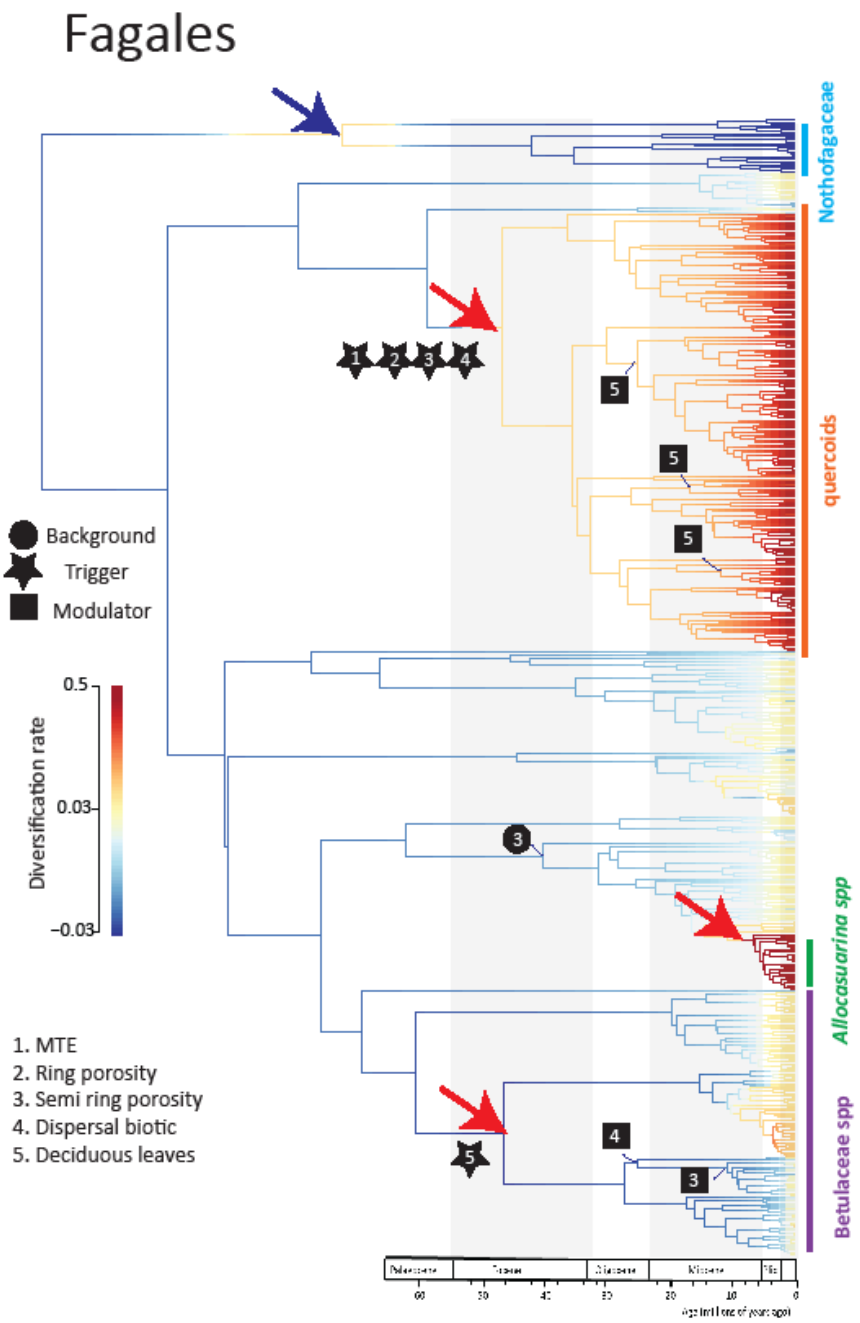

Figure 3
MCC tree from the BEAST analysis for Fagales. Diversification rates are shown along each branch of the phylogeny. Each color section of a branch represents the mean of the posterior density of net diversification rate. The blue (slow down) and red (speed up) arrows indicate where shifts in diversification regime occur. Each number represents one of the variables tested. Fagales exhibits three radiations: the Northern Hemisphere subtropical-temperate quercoids (*Quercus*, *Lithocarpus* and *Castanopsis,* ~923 spp) and the (cold) temperate *Carpinus* (~49 spp) and *Betula* (~97 spp) (i.e. both members of Betulaceae), and a clade of the sclerophyllous Australian *Allocasuarina* (~61 spp). The temperate South American-Australasian *Nothofagus* (~36 spp) shows a slowdown.

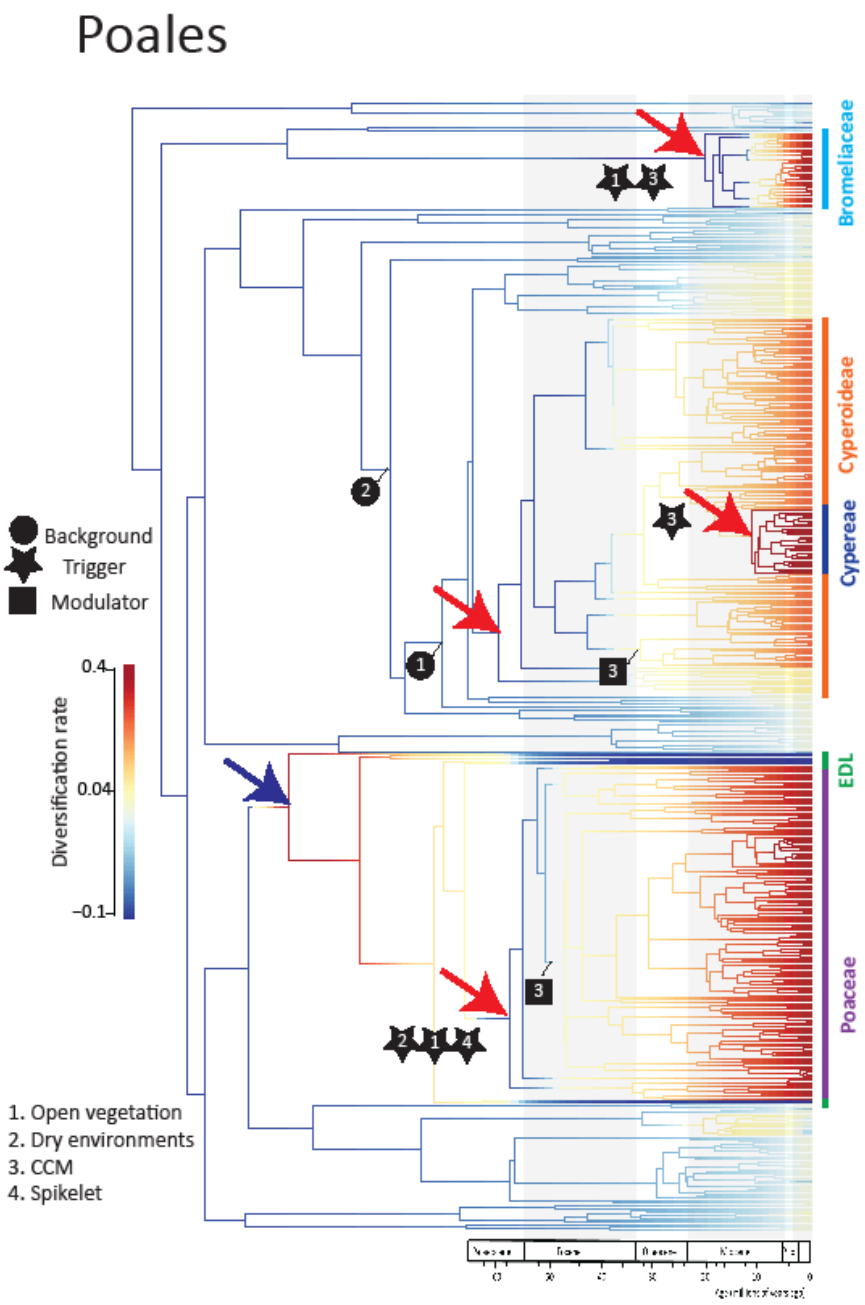

Figure 4

MCC tree from the BEAST analysis for Poales. Diversification rates are shown along each branch of the phylogeny. Each color section of a branch represents the mean of the posterior density of net diversification rate. The blue (slow down) and red (speed up) arrows indicate where shifts in diversification regime occur. Each number represents one of the variables tested. There are four radiations: the South American Bromeliaceae (3,500 spp), the global Cyperoideae (~5,250 spp) followed by a radiation within Cypereae, and the global spikelet clade of the Poaceae (~10,000 spp). The latter is preceded by a slowdown at the base of the graminid clade (~15 spp), which includes Ecdeiocoleaceae, Flagellariaceae, Joinvilleaceae, and the early-diverging lineages ('EDL') of Poaceae.

Table 5
The number of radiations where backgrounds, triggers and modulators (in rows) are (i) extrinsic, intrinsic or both, and (ii) simple or polymorphic, or both (in columns).

	Only extrinsic			Only intrinsic			Both extrinsic and intrinsic			Total radiations with backgrounds, triggers and modulators
	Simple	Polymorphic	Both	Simple	Polymorphic	Both	Simple	Polymorphic	Both	
Background	1	0	1	2	2	1	0	0	2	10/13
Trigger	0	2	0	2	0	0	0	1*	2	7/13
Modulation	0	1	0	0	4	0	0	0	0	5/13

* This cell includes only one radiation, and we report on the trigger variables associated with the Bromeliaceae radiation, which includes open and dry habitats (polymorphic) as extrinsic variable and CCM (CAM) photosynthesis (polymorphic) as intrinsic variable (cf. Table 4).

Table 6
The distribution of retained variables according to their type, timing and roles for the 13 radiations.

	Background		Trigger		Modulator	
	Simple	Polymorphic	Simple	Polymorphic	Simple	Polymorphic
Extrinsic	3	2	0	5	0	1
Intrinsic	8	5	4	3	0	5

Diversification drivers
Eleven of the background variables are simple and seven are polymorphic (Table 6). Among the triggers, four are simple and eight are polymorphic, and all six modulators are polymorphic (Table 6). We found support for the hypothesis that modulators are more likely to be diversification drivers (i.e. polymorphic, labile variables) than triggers and backgrounds (Fisher-exact test: $P = 0.034$). As expected, we also found support for the hypothesis that background variables are more likely to be simple compared to modulators (Fisher-exact test: $P = 0.016$).

Discussion

General
In the three clades we investigated, we found 13 radiations and two slow downs (Figs 2-4). Possibly because of the lack of a detailed sampling (especially within Poales), we did not detect significant shifts in diversification regimes in the more recent histories of the families. Most radiations (10 out of 13) seem to require both extrinsic conditions and intrinsic traits. For all radiations, except for Betulaceae and the quercoids in Fagales and Poaceae and Bromeliaceae in Poales, we located background changes, but only two of these are extrinsic (Table 5). In six radiations we found no triggers. Two radiations were found to be triggered by intrinsic traits, two by extrinsic traits, and three by a combination of intrinsic and extrinsic variables (Tables 2-4). In only five of the 13 radiations we found modulators (Table 5). As predicted, variables that arise late relative to the start of the radiation are more likely to be polymorphic, and so are more likely to stimulate diversification (e.g. either by allowing coexistence after speciation, or by driving divergence during speciation) rather than persistence in a particular environment (extrinsic) and/or morphology (intrinsic).

Although BiSSE may currently be the best method to filter out variables not linked to diversification rate shifts, it is possible that some discarded variables, even though not significantly associated with high diversification rates across the whole phylogeny, may still be potential causes of

radiation for a specific clade. Therefore, we included the classification of those variables relative to radiations (Tables 2-4; see Supporting Information Fig. S2).

There is no biologically objective way of locating radiations. BAMM is most likely the best tool, but if the species sampling ratio is given only at nodes which are very deep in the tree it may shift the node of the diversification regime shift further in the past than where it should be, and it could miss nested radiations. Consequently, there may be nested radiations in our study clades that have not been detected because of sparse taxon sampling and the assignment of unsampled diversity to higher taxonomic levels. This could account for the difference between our radiation shifts in Bromeliaceae and those postulated by Givnish et al. (2014). Finally, the methods used for molecular dating (fossil assignments, prior distributions and rate correction methods) may impact our results. However, since the temporal classification of variables is relative to rate shifts obtained on the same chronogram, such dating errors will not impact our results.

Intrinsic and Extrinsic conditions

Theoretically, a radiation needs both extrinsic conditions and intrinsic traits except when it occurs in a geographically isolated and non-competitive habitat. Extrinsic conditions and intrinsic traits may be complex in several ways, making it difficult to identify the underlying and effective causes of these conditions.

Across the three clades we found that shifts in extrinsic conditions often triggered the radiation (shifts to mountains in two out of six Ericaceae radiations, a shift to Mediterranean-type ecosystems in the quercoid, and shifts to open and dry vegetation in the Poaceae and Bromeliaceae radiations), as well as being the background (open and dry vegetation in the Cyperoideae and the Cypereae, shifts to mountain in *Rhododendron* 2 and *Gaultheria* in the Ericaceae) or the modulator (mountains in *Rhododendron* 1). Thus, ecological opportunities may influence radiations in general even if the initiation of the radiation happens later, or if the opportunity arises after the initiation of the radiation.

Past climate change is often proposed as a trigger for radiations. This is usually based on a three-part argument. The first is biome or habitat change optimized over a phylogeny and modeled to have taken place at a particular node. In the second part, this node, if on a time-calibrated phylogeny, provides an estimate of the time of this change. In the third part, this dating is used to provide a link to global climate changes. This logic has been used to link the radiation of succulent plants (Arakaki et al. 2011), cycads (Nagalingum et al. 2011), and grasslands (Jacobs et al. 1999) to the 'modern planet' climate established ca. 14 Ma by the Antarctic glaciations (Zachos et al. 2001), which resulted in widespread seasonal dry climates. Such global climate-driven 'turnover events' (Vrba 1985, 1993) possibly also occurred at the Eocene Optimum and the Eocene-Oligocene transition. In Poales, the radiations of Cyperoideae and the spikelet clade of Poaceae occurred at 50 and 55 Ma, respectively, suggesting that the Eocene Optimum may have acted as an extrinsic factor for these two radiations. More likely, the establishment of seasonal monsoonal climates (Huber and Goldner 2012) in the Late Eocene might have caused the establishment of more open vegetation into which these two Poalean clades radiated.

In our analyses, extrinsic potential causes of radiations were often associated with intrinsic changes. Across the three clades, we found that shifts in intrinsic traits were also potential triggers for radiations (ring porosity and biotic dispersal in the quercoid radiation, deciduous leaves in the Betulaceae radiation, CAM photosynthesis in Bromeliaceae, and a spikelet in the Poaceae radiation), as well as backgrounds (low SLA and a shrubby growth-form in all radiations in Ericaceae, semi-ring porous wood in Fagales), or modulators (deciduous leaves in quercoids, biotic dispersal and semi-ring porous wood in Betulaceae and C_4 photosynthesis in the Cyperoideae and Poaceae radiations) (Figs. 2-4). Our method shows that intrinsic traits are much more important in determining the backgrounds

for radiations than suggested by the Simpsonian (1953) model. This model implies that the environment existed, waiting for lineages either to reach it (physical access or dispersal) or evolve suitable traits to enter it. Ericaceae present a complex situation. Here the background is probably the combination of ericoid roots, low SLA leaves, and a habitat on oligotrophic soils (Read 1996). Our analysis could not disentangle this set of interactions as the whole family shows this syndrome, and our analysis starts only at family level. However, since the radiations are within the family, this syndrome acts as background for these radiations. A comparable situation was reported in the succulent clades of the Caryophyllales, where succulence evolved before the radiations started (Arakaki et al. 2011); presumably this was in response to locally dry habitats. The actual radiations were triggered by Late Miocene aridification and the evolution of more extensive areas of seasonal climate. Lieberman (2012) suggested that these should be called 'exaptive radiations' as the trait evolved before the extrinsic condition. However, in both Ericaceae and Caryophyllales, the trait syndromes (ericoid mycorrhizae, low SLA leaves, and succulence) evolved in response to the conditions in which they still function. The trigger of the radiation was the expansion of these habitats by orogeny (Ericaceae) or climate change (Caryophyllales). Consequently the term 'preadaptation' might be more suitable.

The roles of extrinsic and intrinsic variables in a radiation are complex and seem to be intermingled, especially because plants modulate the environment (Linder et al. 2012) and can change the extrinsic conditions not only for themselves but also for other organisms. This complex interaction may well have been of central importance in the Poaceae radiation, and also for the Cyperoideae. Poaceae diversification slowed down significantly in closed vegetation (the graminid diversification dynamics change), and accelerated in open habitats (the spikelet-clade radiation) (Fig. 4). In the latter case, grasses and their intrinsic traits created part of the extrinsic environment, the open vegetation. The evolution of angiosperm forests in the late Cretaceous also acted as biotic modifier (Linder et al. 2012) of the environment (cool, shady) by providing new food and new chemical resources. This has been shown to be linked to the radiation of epiphytic ferns (Schneider et al. 2004) and ants (Moreau et al. 2006). The radiations in forest understory plants such as *Impatiens* (Janssens et al. 2009) and *Begonia* (Thomas et al. 2012) were only possible in the cool shade of the tropical forests. The transformation of forests to savannas may have been facilitated by grasses fuelling fire, and the expansion of grassland is associated with an increase in fire frequency (Bond et al. 2003, Bond et al. 2005, Beerling and Osborne 2006, Hoetzel et al. 2013). This modulation complicates the simplistic distinction between intrinsic and extrinsic variables.

Although radiations occur in a context in which multiple variables are involved, theoretically, each radiation should be triggered by a single variable. This may be the last element of a trait syndrome, or the actual colonization event of a new biome. However, we cannot resolve a singular trigger in three cases (Tables 2-4), and there could be several reasons for this. Firstly, where the radiation is subtended by a long branch (such as the stem of Bromeliaceae; Fig. 4) the sequence of changes along these branches cannot be resolved, and so background changes cannot be separated from triggers. Secondly, because we start with the assumption that all variables are triggers, if the dating has a wide variance this assumption cannot be rejected. In this case, variables that should be background or modulators are still listed as triggers. Finally, the lack of resolution could also be real. Roquet et al. (2013) show that the cushion growth form is not only an adaptation to alpine conditions, but is linked to the radiation in *Androsace* (Primulaceae). They suggest that this growth form change occurs as the populations are elevated by orogeny selecting for adaptation to the increasingly harsh alpine conditions. In this case it is possible that the extrinsic condition (mountains) and intrinsic trait (cushion growth form) evolve at the same time, as 'compound triggers'.

Diversification drivers

Several of the intrinsic and extrinsic traits identified as triggers or as modulators might function as diversification drivers, particularly if they are polymorphic within the radiation. These may include mountains in the Ericaceae radiations (triggers in the Richeeae, the Himalayan *Rhododendron* 2, and Vaccinieae radiations; Fig. 2; Table 2; see Schwery et al., 2015). In the Fagales, these include biotic dispersal and semi-ring porosity in the Betulaceae radiation, and Mediterranean-type ecosystems, ring porosity and leaf deciduousness in the quercoid radiation (Fig. 3; Table 3). Open and dry vegetation can be included for the Poaceae and the Bromeliaceae radiations, and the C_4 and CAM photosynthesis for the Cyperoideae, the Poaceae and the Bromeliaceae radiations in Poales (Fig. 4; Table 4). The predominance of polymorphic variables among the modulators, and their total absence from the background variables, is consistent with polymorphic variables functioning as diversification drivers.

Radiation modulators, which arise after the start of the radiation, include one extrinsic condition and four intrinsic traits (Table 5). The shift to mountain habitats in *Rhododendron* 1 functions as a modulator (Table 2), which implies that the ancestrally low SLA leaves and the shrub habit may have been pre-adaptations for colonizing mountain systems. Some of the traits might constitute further adaptations to the environment, and as such can be seen to 'fine-tune' an adaptive radiation. This is illustrated by the CAM photosynthesis and absorbtive trichomes in Bromeliaceae, allowing the plants to deal with short-term dry habitats (Givnish et al. 2014, Silvestro et al. 2014). A comparable situation is found in the Poaceae. Both cold-tolerance, which was possibly the key to the north-temperate radiation of the Pooideae (Edwards and Smith 2010), as well as C_4 photosynthesis (Edwards and Smith 2010, Spriggs et al. 2014) evolved as modulators after the radiation of the spikelet clade. The evolution of such modulators is important to maintain the radiation, and generate a set of nested radiations which give the impression of a single large radiation.

We did not take into account the complexity of traits. Traits with many different states, such as the different shapes of floral spurs, may also be effective in stimulating diversification. Other traits which may stimulate diversification could include mechanisms that allow a more precise mate recognition, such as the dichromatism in cichlids (Wagner et al. 2012) and so limit gene flow. Polyploidy in plants (Soltis et al. 2009) and chromosomal evolution caused by diffuse centromeres in Cyperaceae (Givnish et al. 1999) may act as drivers of post-mating reproductive barriers and therefore may stimulate diversification. Flowers and their rich diversity of form are often linked to more precise pollen transfer and so indirect specific mate recognition (van der Niet and Johnson 2012). Diversification may simply be a side effect of more space available. If this is the case, then climate change creating extensive open habitat may also have been the speciation mechanism in many of the radiations we documented – for example the *Betula* radiation into the extensive cold northern Late Miocene habitats and the grass and Cyperoideae radiations into the new open seasonally dry pyrophytic Late Miocene habitats. The montane radiations in Ericaceae may have been the result of the fragmented montane habitats and the steeper divergent selective gradients. Testing such hypotheses remains quite challenging.

It would be difficult to assemble the complete list of traits and environmental conditions that influence radiations, as it requires an exhaustive search through trait-space. The method we used here starts with prior hypotheses of potential traits, which are then tested. Consequently it is not surprising if more detailed studies, such as that of Givnish et al. (2014) find additional traits.

Conclusion

Classifying the variables into three attributes allowed us to explicitly distinguish the roles of extrinsic conditions and intrinsic traits in a radiation. Firstly, we were able to test the validity of the Simpsonian model (extrinsic variables set up the ecological opportunity and intrinsic traits start and modulate the

radiation). Secondly, this framework allowed us to infer diversification scenarios. Finally, this protocol may be relevant for identifying variables (extrinsic vs. intrinsic) and testing whether backgrounds, triggers and modulators were selected. It is very likely that not all variables linked to the radiations were sampled in our study and this could, for example, account for the lack of extrinsic conditions acting as backgrounds in Fagales. Our novel classification permits the *a posteriori* selection of variables that may play roles as backgrounds, triggers and/or modulators in the radiation of a group of organisms.

Using the paradigm that variables linked to radiations can be sorted in three classes leads to a much better insight into the conditions and traits that might be contributing to radiations. Contrary to expectations, we show that both extrinsic conditions and intrinsic traits are involved with setting up the background conditions for radiations to take place as well as triggering them, and possibly modulating ongoing radiations. Finally, diversification drivers can be recognized by always being polymorphic. The triggers of radiations can be decomposed into types, timing and roles and this could enhance our understanding of the processes that lead to the generation of the extraordinary diversity of organisms.

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Supporting Information Chapter VI

Methods S1 Traits (extrinsic and intrinsic) coding and BiSSE diversification models that were tested for Ericaceae, Fagales and Poales.

For Ericaceae, the extrinsic (mountain association) variable was scored for each species by mapping the distributions inferred from the GBIF data over the mapped mountains of the world (Körner *et al.*, 2011). Species were assigned to being "montane" when at least 70% of the distribution was in mountainous areas (Schwery *et al*., this issue). Intrinsic variables (evergreen/deciduous, epiphytism, growth forms: tree, shrub and herb) were scored from online databases (http://florabase.dec.wa.gov.au; http://plantnet.rbgsyd.nsw.gov.au; http://plants.usda.gov/plantguide/; http://www.efloras.org; http://www.missouribotanicalgarden.org/PlantFinder/; http://www.nybg.org/bsci/res/lut2/; accessed November 2013) and the literature (Small 1903, Rydberg 1954, Salmon 1968, Wagner et al. 1990, Davidian 1992, Castroviejo et al. 1993, Pennington et al. 2004, Lauber et al. 2012). Specific leaf area (SLA) data were in part obtained from the TRY database (Kattge et al. 2011), in part from our own measurements from fresh as well as herbarium specimens by dividing leaf area of individual leaves by their dry-weight after (re-) drying the leaves in the oven for sufficient amount of time, for details see Schwery et al. (2015).

For Fagales, extrinsic variables (Temperate Deciduous Forests (TDF), Tropical Evergreen Forests (TEF), Mediterranean-type ecosystems (MTE) and open tropical systems) and intrinsic variables (evergreen/deciduous leaves, ring/semi ring/diffuse porosity, biotic dispersal, Nitrogen-fixation, serotiny and scale leaves) were scored from the publicly available online databases of Encyclopedia of Life (NatureServe Explorer 2002) (NatureServe, 2002), the inside-wood database (http://insidewood.lib.ncsu.edu, InsideWood 2004 onwards) and from the literature (Tutin 1980, Watson and Dallwitz 1992 onwards, Flora of North America Editorial Committee 1993, Kubitzki 1993, Wu and Raven 1999). Assignation to biomes was done by mapping species distributions inferred from GBIF (Flemons et al. 2007) specimen data (http://www.gbif.org/, December 2013) over Köppen climate maps (Kottek et al. 2006). The assignations were in addition compared to published flora accounts.

For Poales, extrinsic (wet/dry and open/closed habitats) and intrinsic (CO_2-concentrating mechanisms, presence of grass spikelets, presence of water tanks) variables were all scored from the literature (Bew 1929, Moldenke 1955, Ake-Assi 1963, Benzing 1980, Lazarides 1980, Dahlgren and Rasmussen 1983, Renvoize 1984, Holst 1997, Kubitzki 1998, Smith and Till 1998, Clayton et al. 2006).

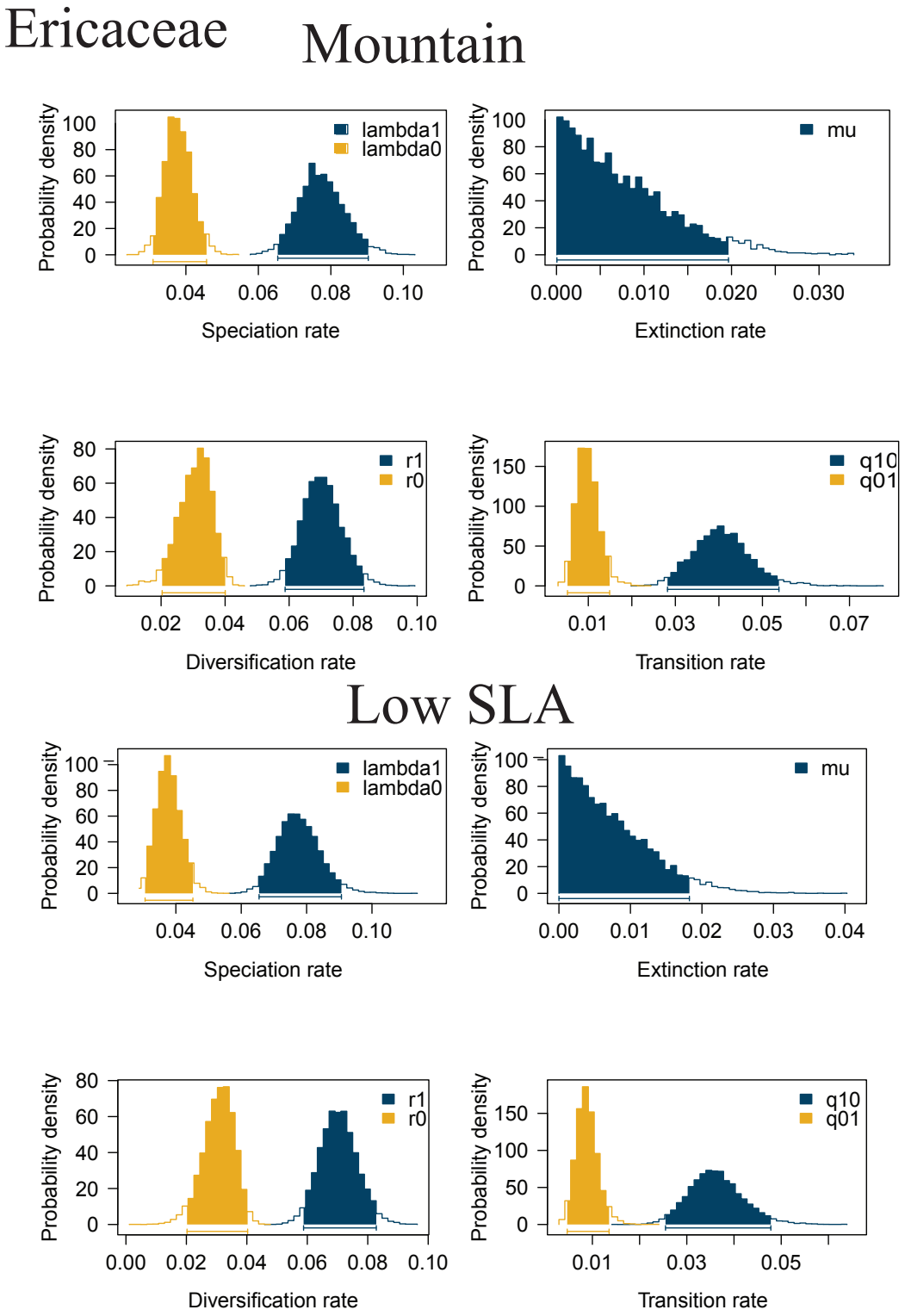
For all three clades, we used BiSSE (Maddison et al., 2007) to test whether variables were associated with different diversification dynamics. Because our sampling was incomplete, BiSSE calculations were corrected with a function (*sampling.f*) that incorporates the known number of missing species assigned to a particular state for each coded trait. This was only possible for the Poales because the variables selected were available for all species from the litterature. However, the variables selected for Ericaceae and Fagales were not, and it was not possible to estimate a proportion of missing species for each state of the variables. We compared the fit of seven models (Table S1), and selected the best model with a likelihood ratio-test and AIC scores. The best model was then used to estimate the parameters (cf. Fig. S1).

Table S1: Seven BiSSE models with different constraint settings and degrees of freedom (df).

	BiSSE models						
Models	λ_0	λ_1	μ_o	μ_1	q_{01}	q_{10}	df
I	$\lambda_0=\lambda_1$				$q_{01}=q_{10}$		4
II	$\lambda_0=\lambda_1$		$\mu_o=\mu_1$		$q_{01}=q_{10}$		3
III	$\lambda_0=\lambda_1$		$\mu_o=\mu_1$				4
IV	$\lambda_0=\lambda_1$						5
V					$q_{01}=q_{10}$		5
VI			$\mu_o=\mu_1$		$q_{01}=q_{10}$		4
VII							6

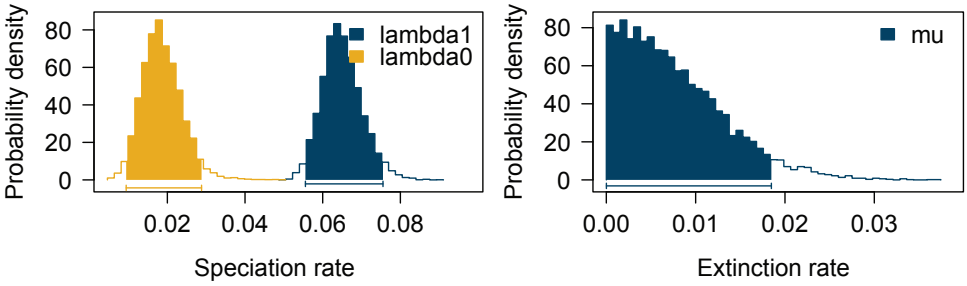
'0' and '1' refer to absence and presence, respectively, for each variable tested. λ, μ and q represent speciation, extinction and transition respectively.

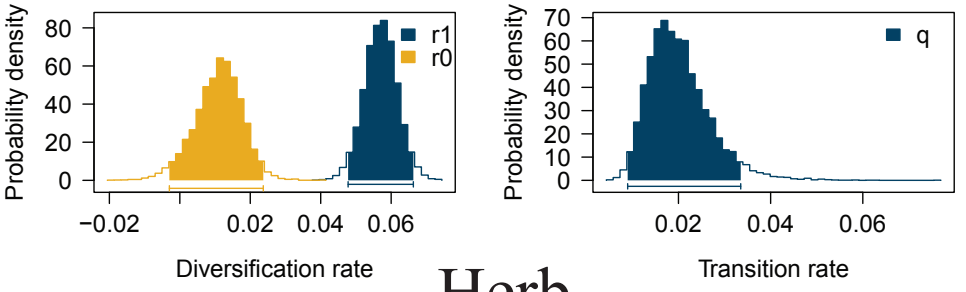
Figure S1 Posterior probability distributions for Ericaceae, Fagales and Poales of the best BiSSE model for variables that were associated with higher diversification rates. The bars at the bottom of the distributions and the shaded areas correspond to the 95% credibility intervals. Lambda, mu, q and r indicate speciation, extinction, transition and net diversification rates, respectively. '0' and '1' represent absence and presence of the variables tested. If only one posterior density is shown, this means that a model with equal rates between states was selected for the parameter of the trait.



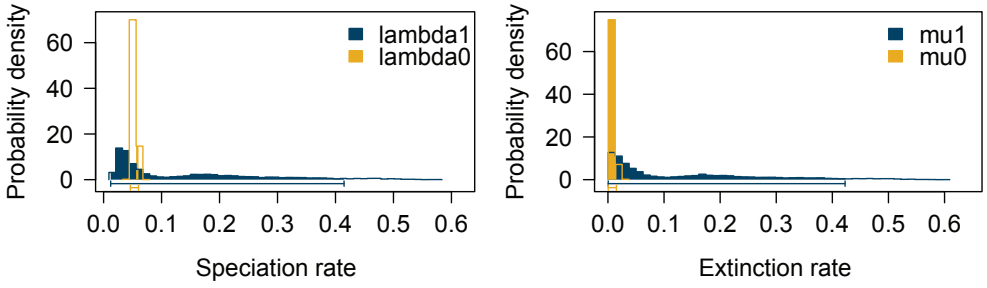
Ericaceae

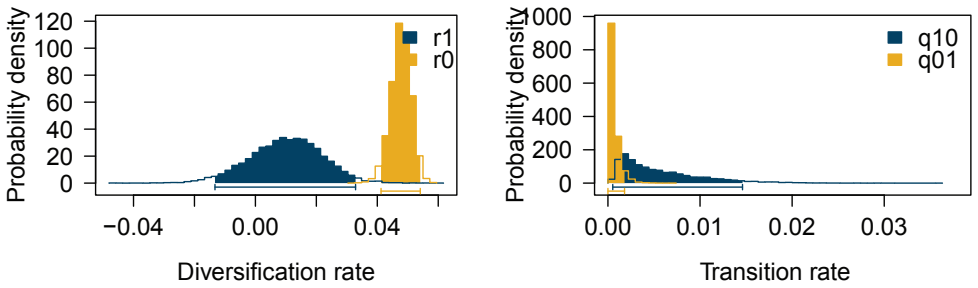
Shrub



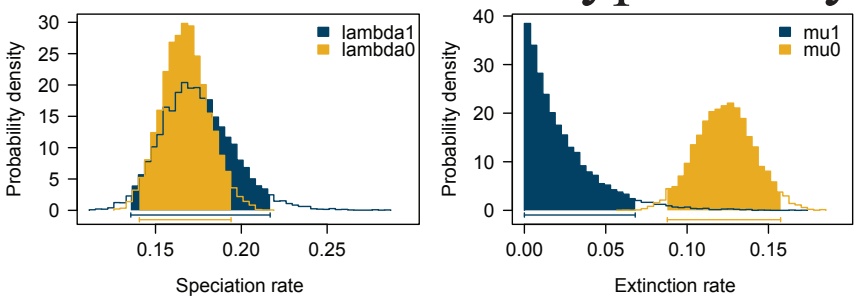


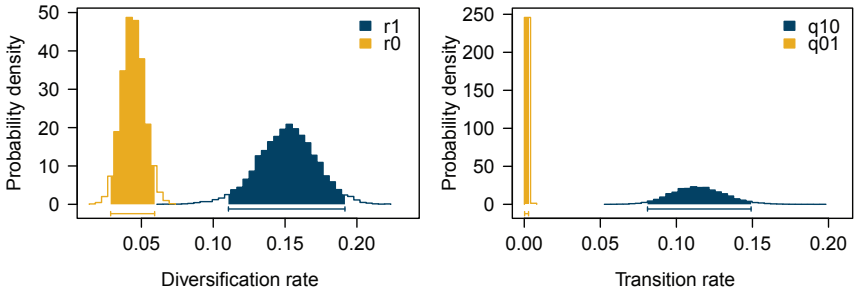
Herb



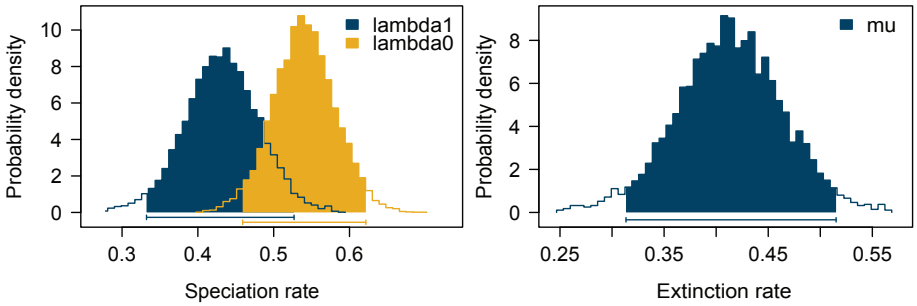


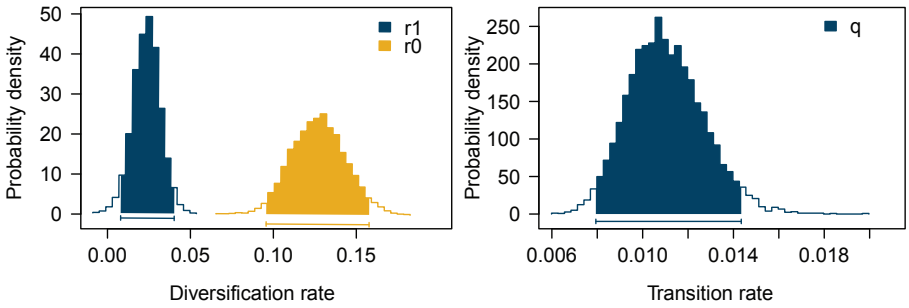
Fagales Mediterranean-type Ecosystem





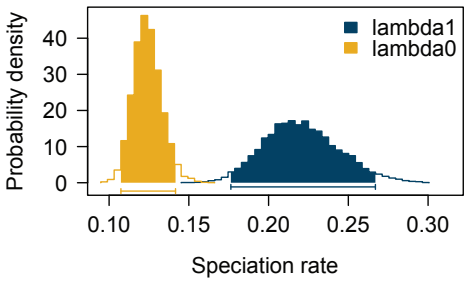
Tropical Evergreen Forest

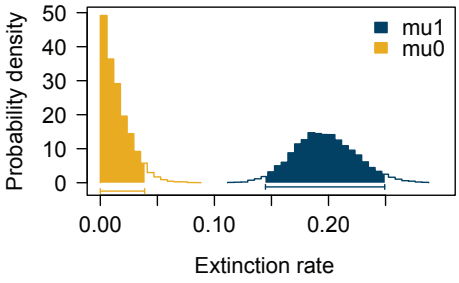


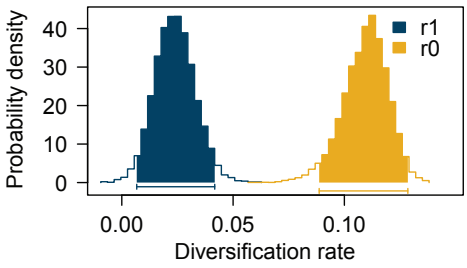


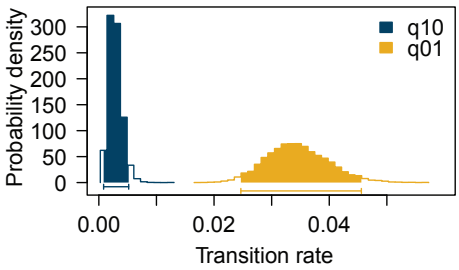
Fagales

Temperate Deciduous Forest

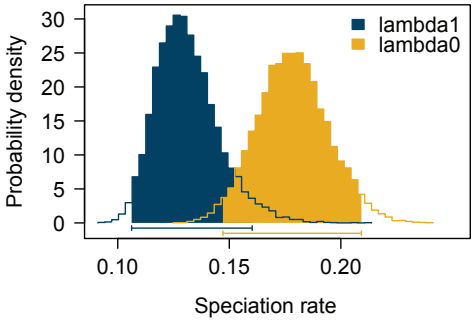


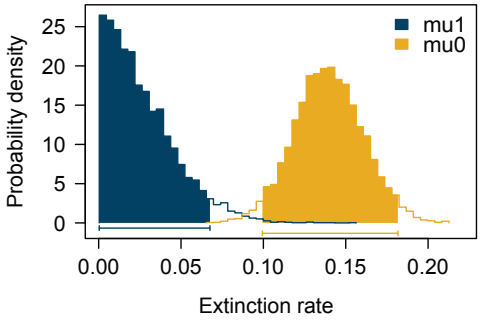


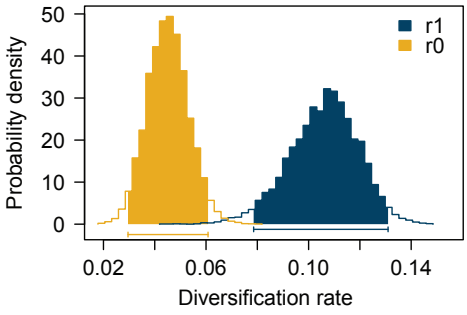


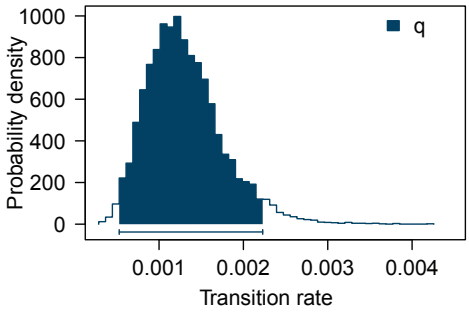


Ring porosity



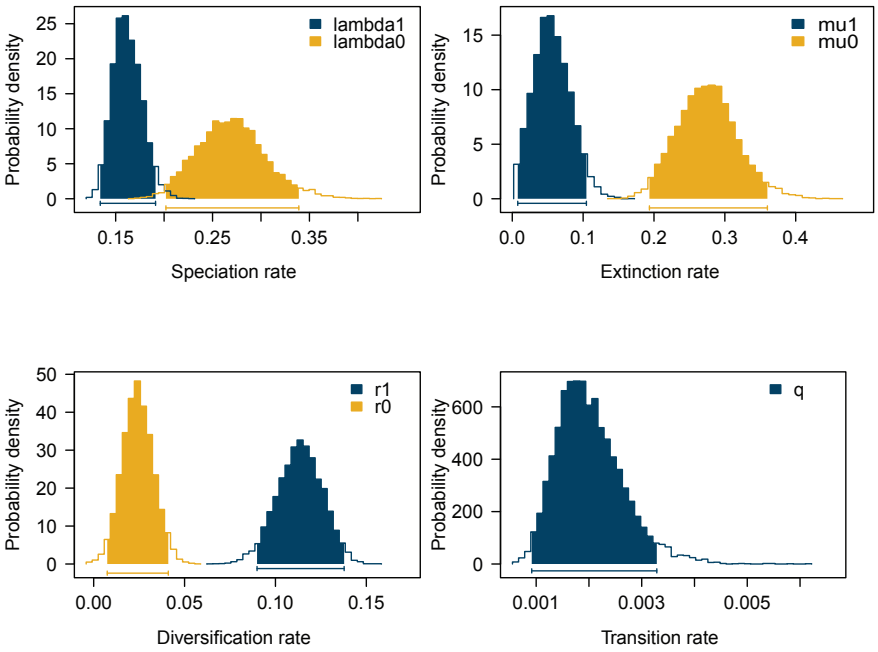




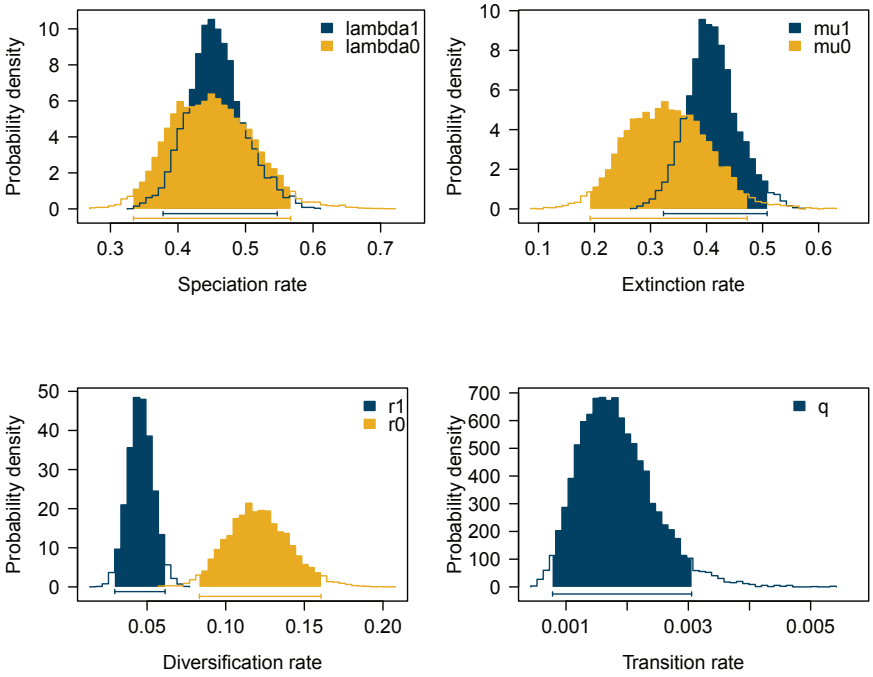


Fagales

Semi-ring porosity

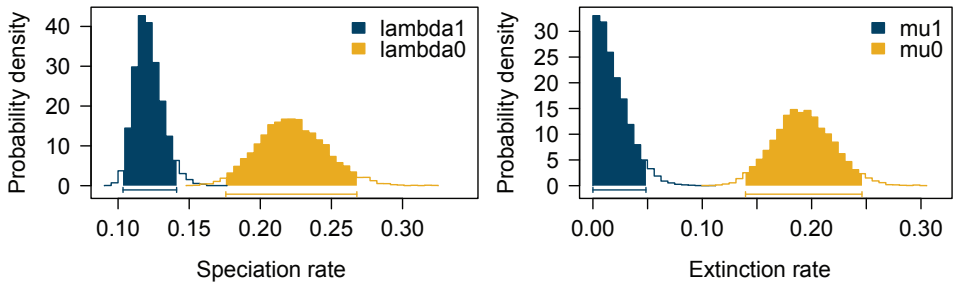


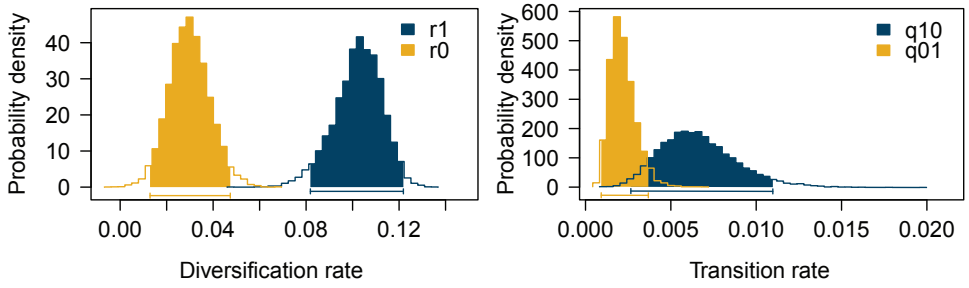
Diffuse porosity



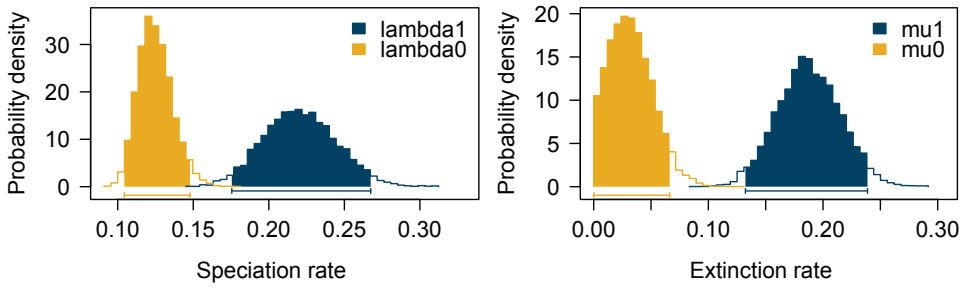
Fagales

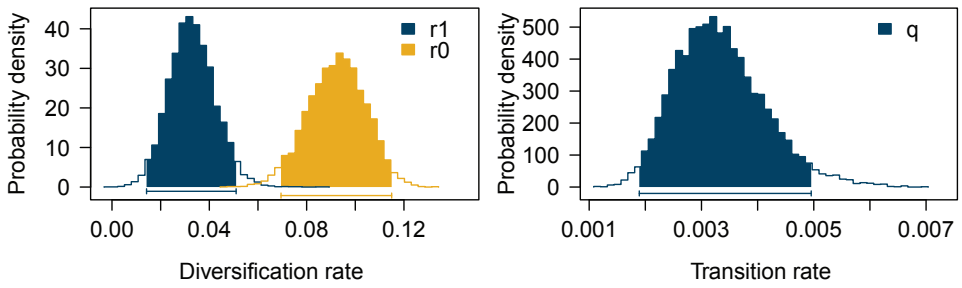
Biotic dispersal





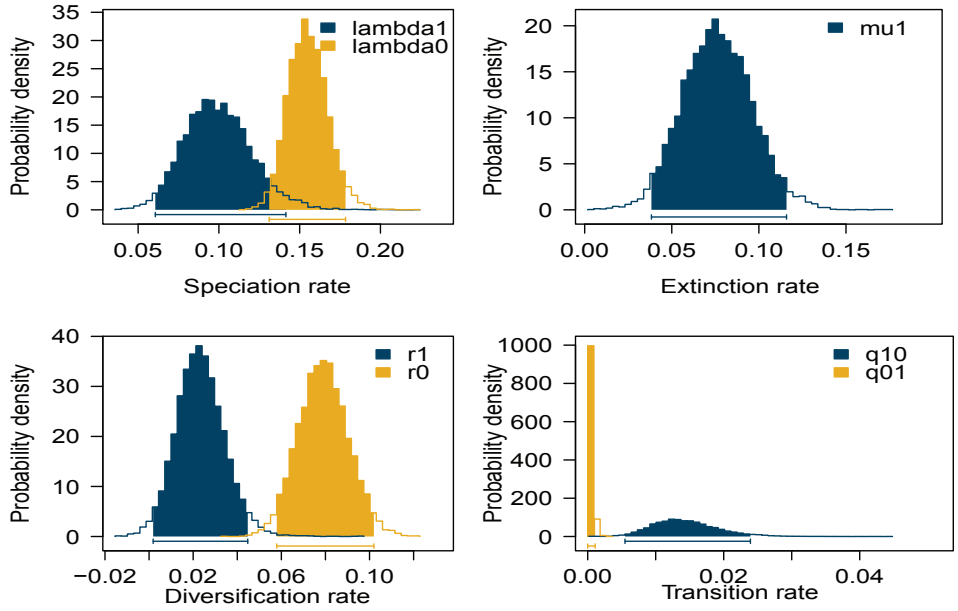
Abiotic dispersal



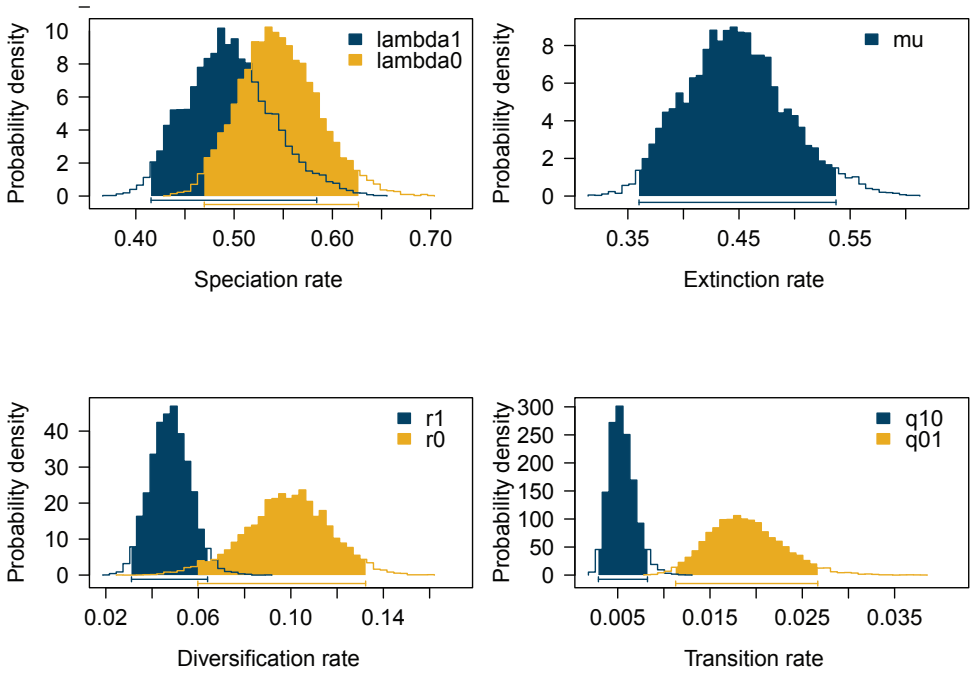


Fagales

Passive dispersal

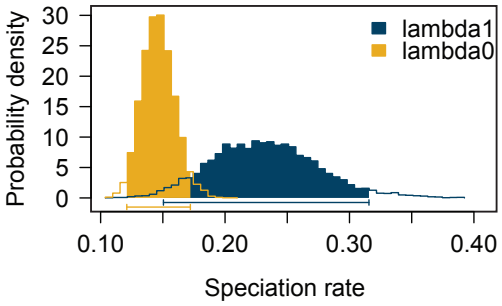


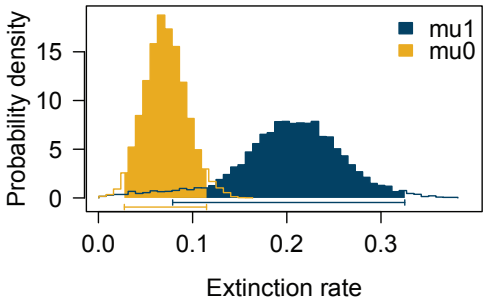
Evergreen leaves

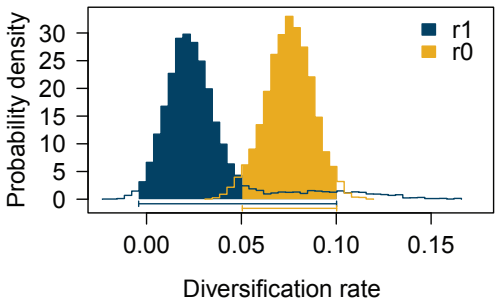


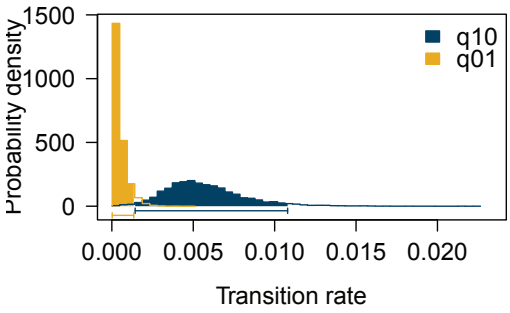
Fagales

Nitrogen fixation

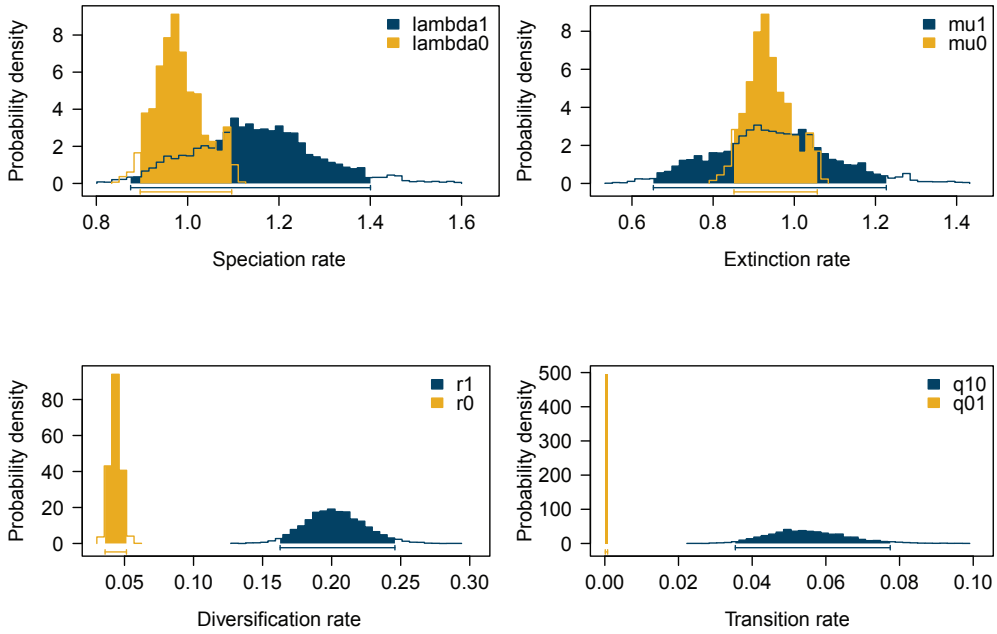




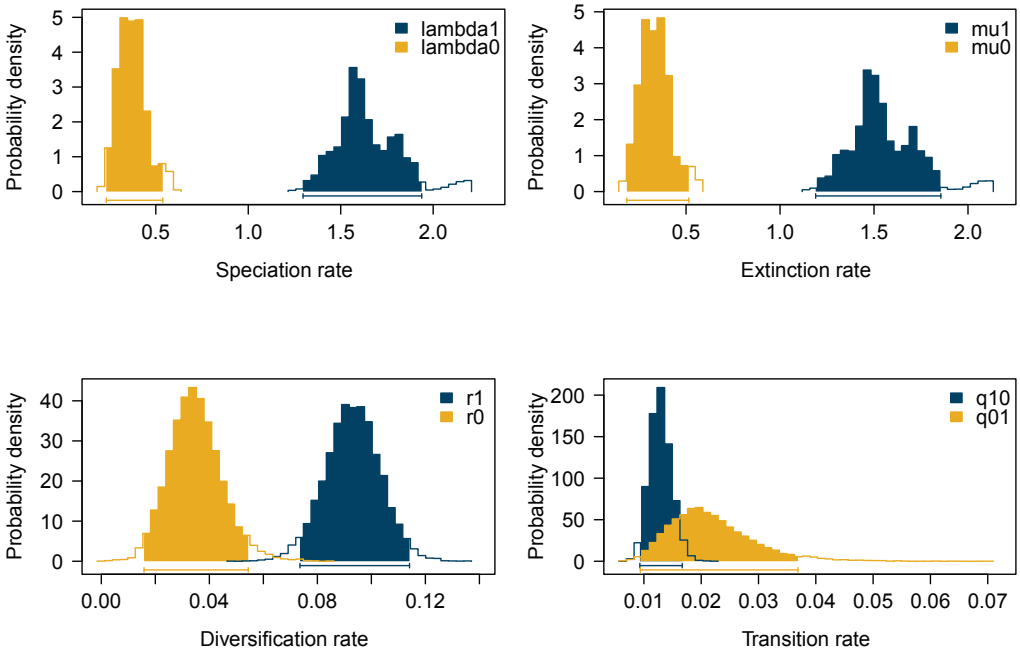




Poales Open vegetation

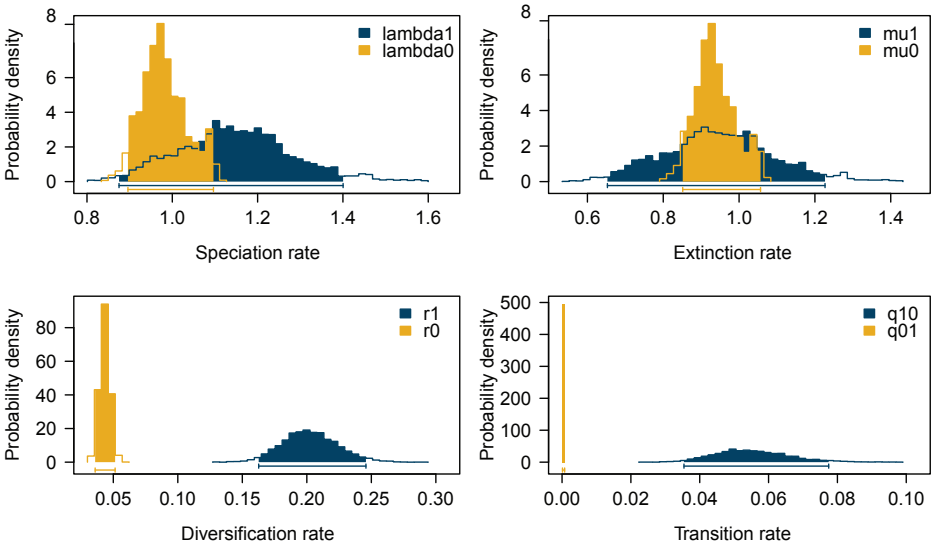


Dry environments



Poales

C4 + CAM photosynthesis



Spikelet

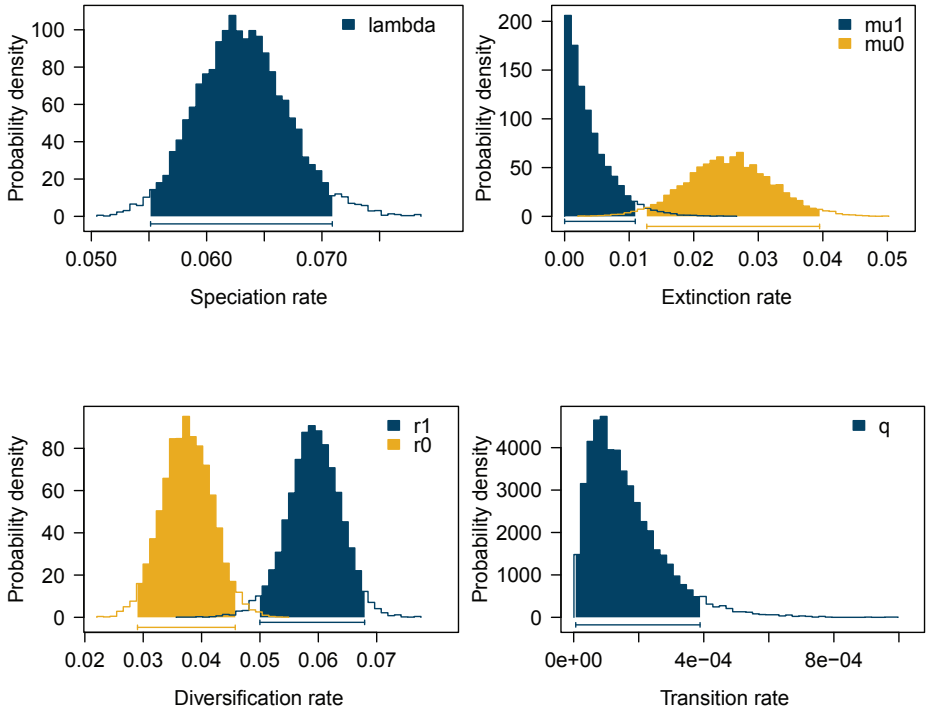
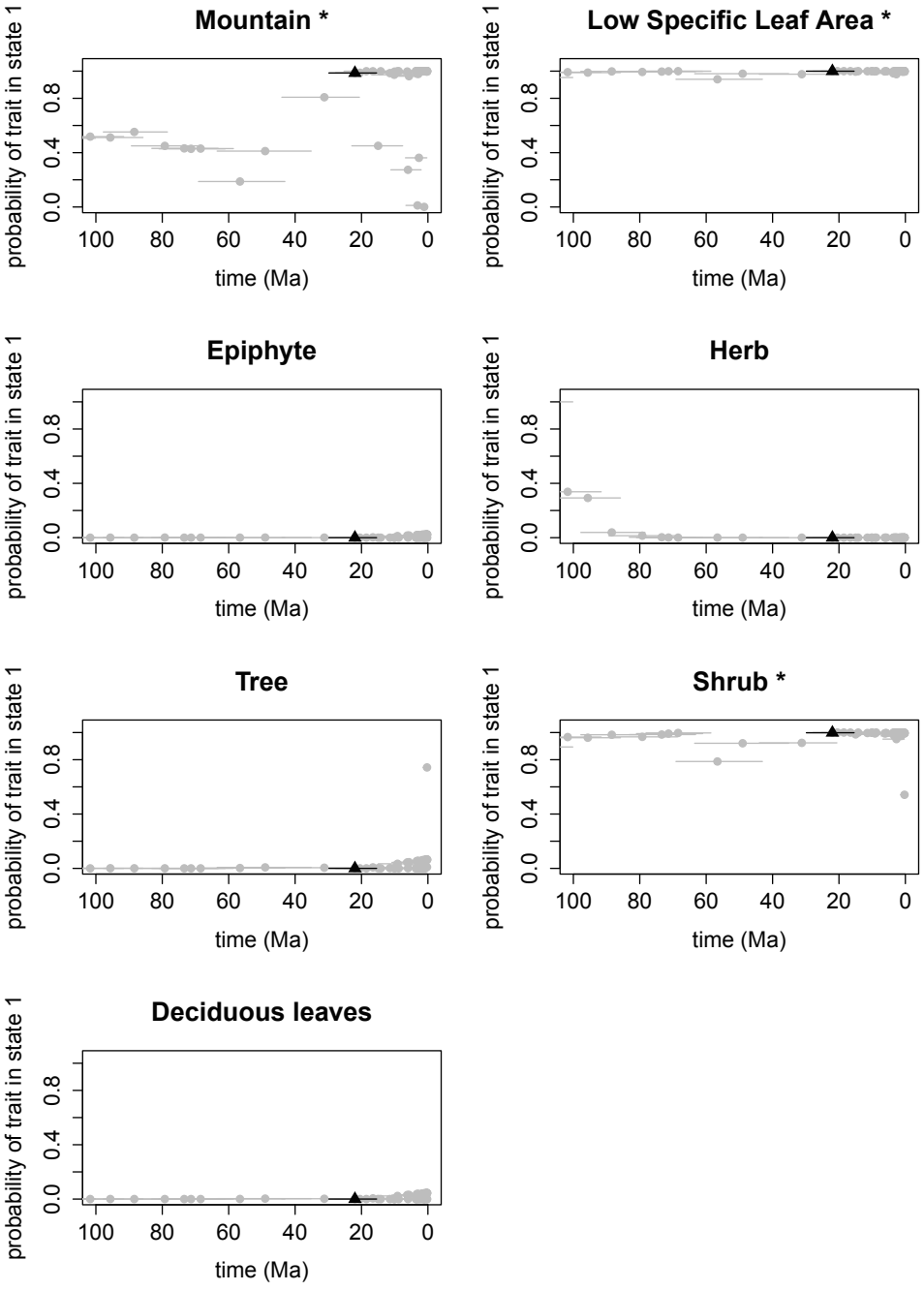
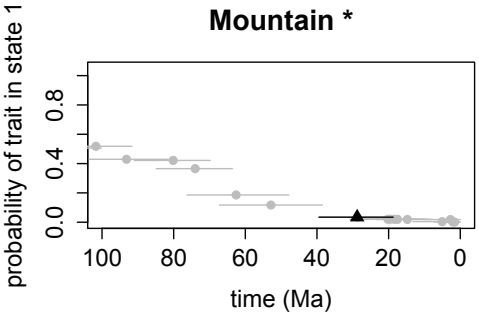


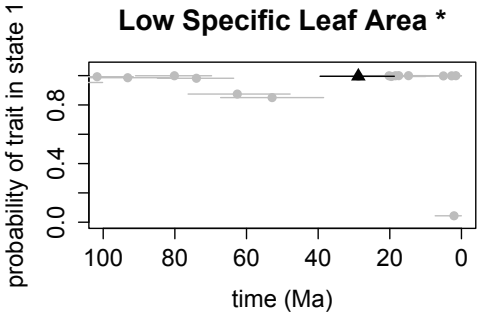
Figure S2 Temporal classification of the variables for Ericaceae, Fagales and Poales following the protocol described in Material and Methods and in Fig. 1. Black triangles represent the node at which the radiation occurred. The horizontal bars indicate the 95% HPD intervals.

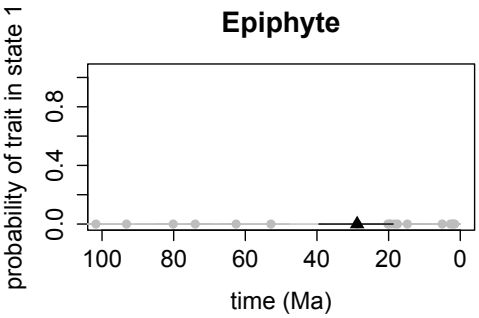
Ericaceae: Richeeae

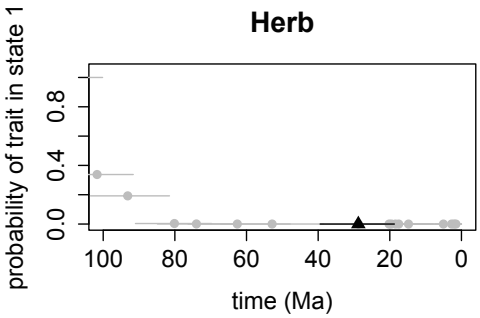


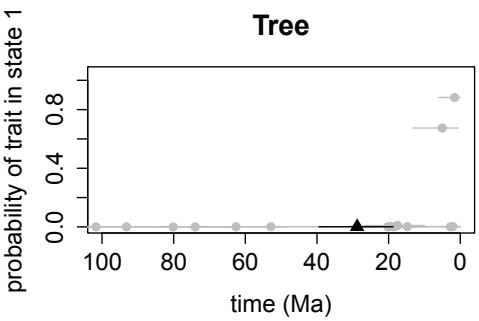
Ericaceae: *Erica*

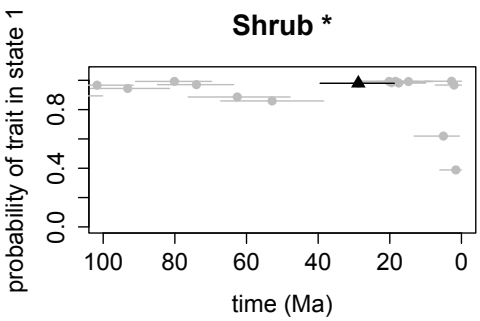


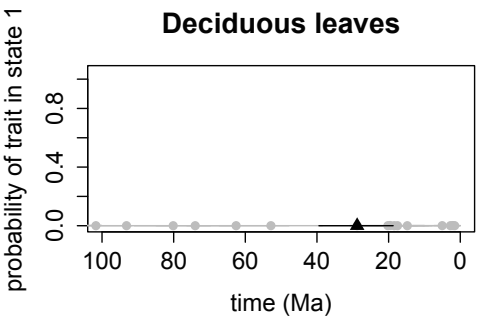




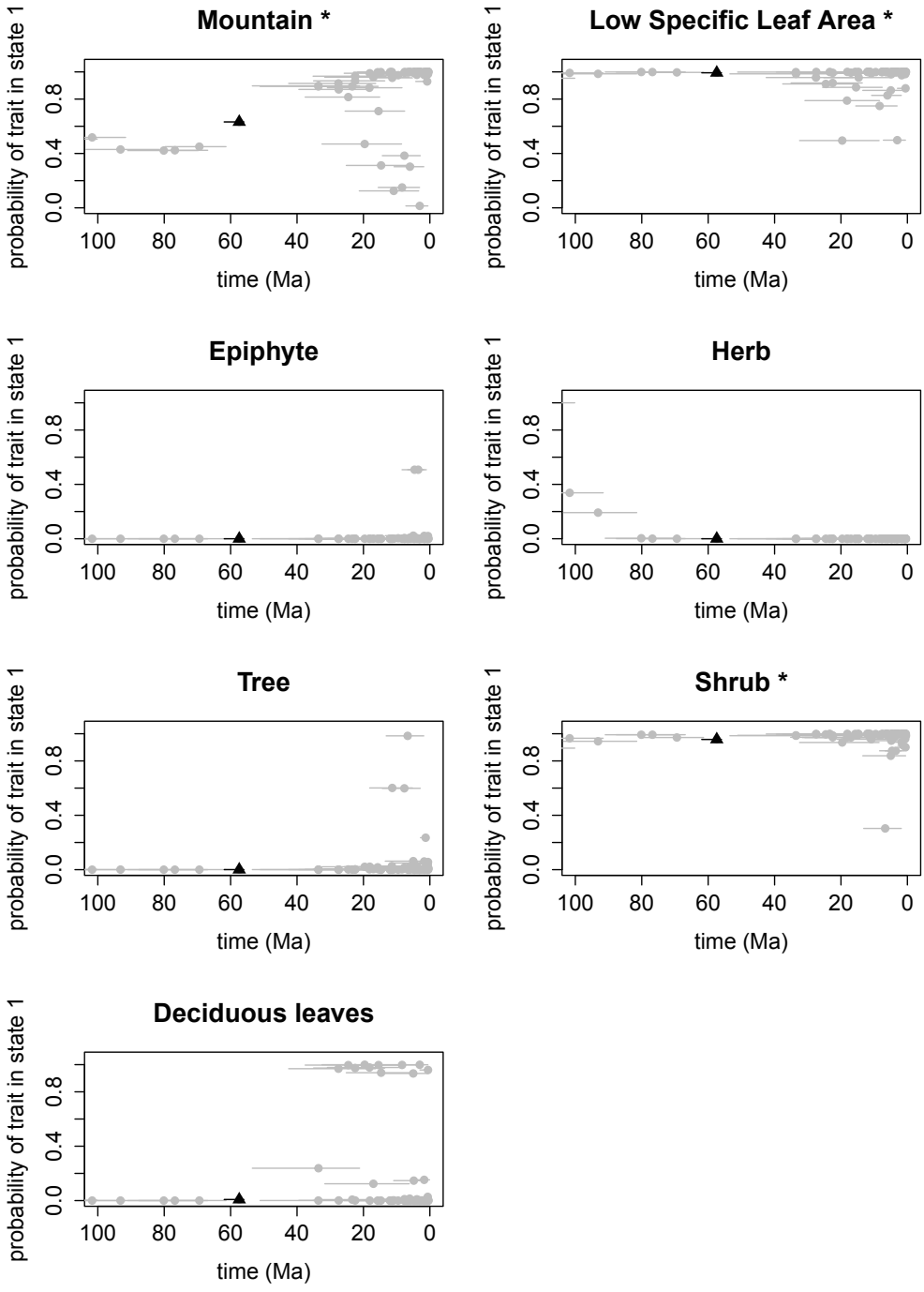




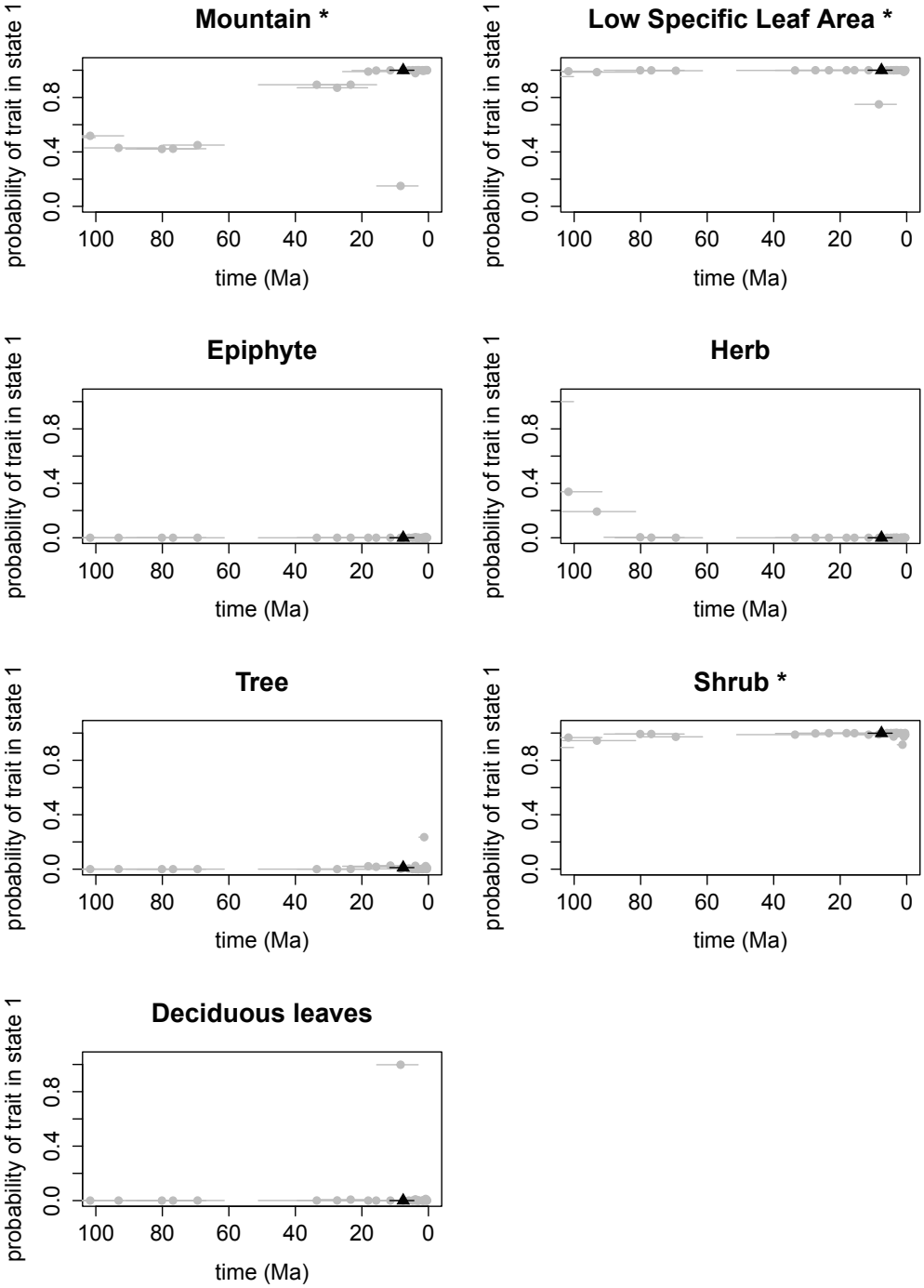




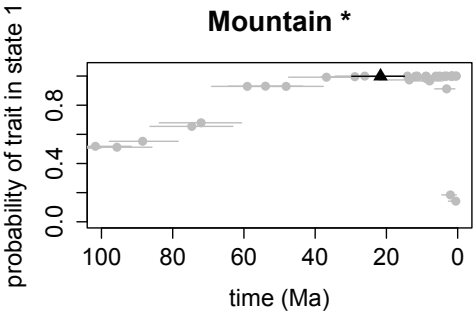
Ericaceae: *Rhododendron 1*

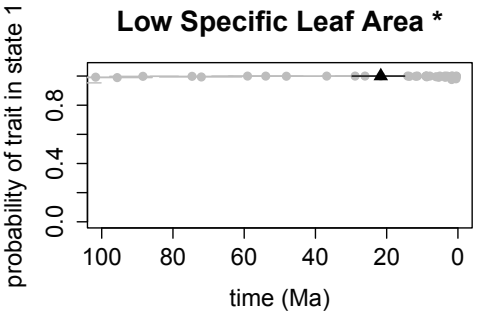


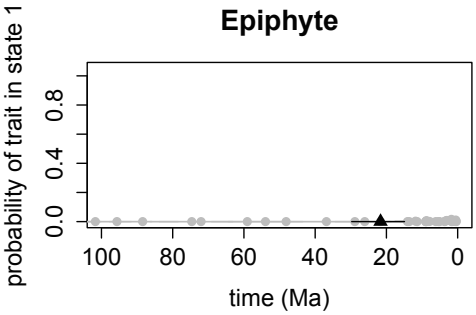
Ericaceae: *Rhododendron 2*

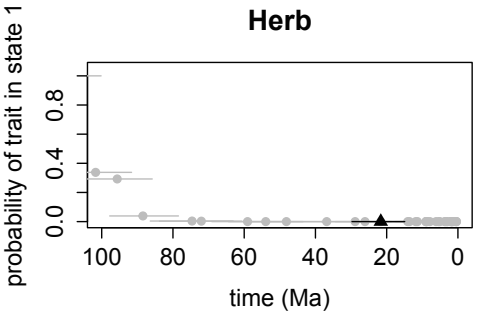


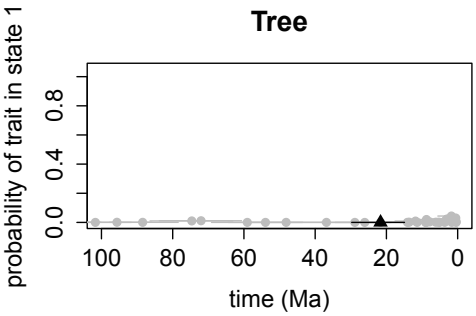
Ericaceae: *Gaultheria*

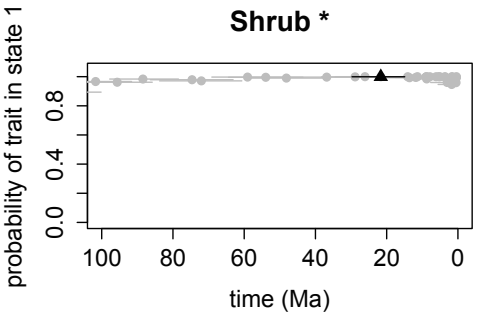


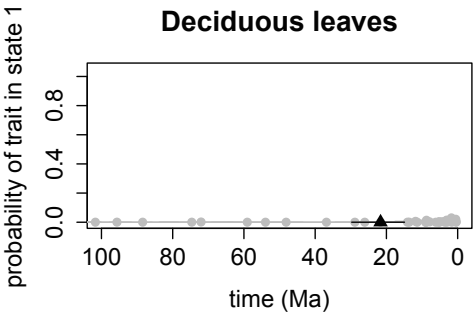




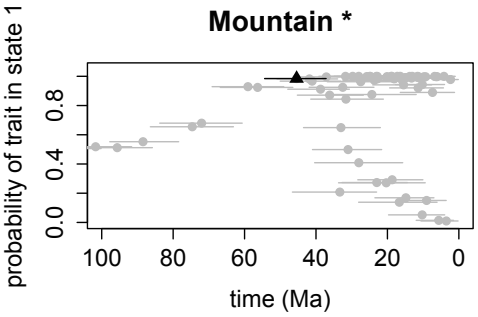


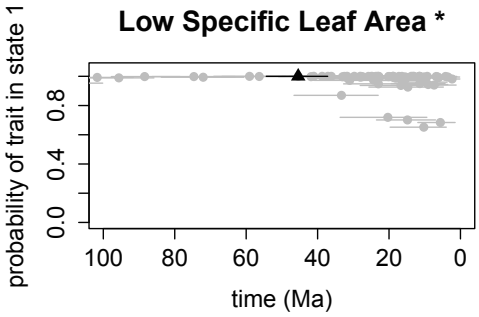


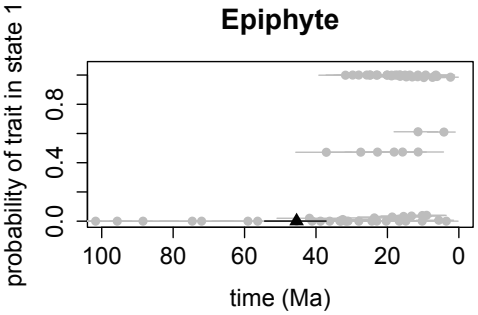


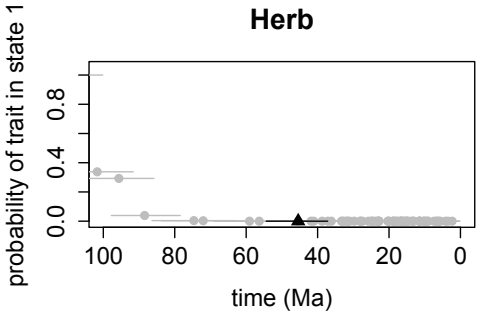


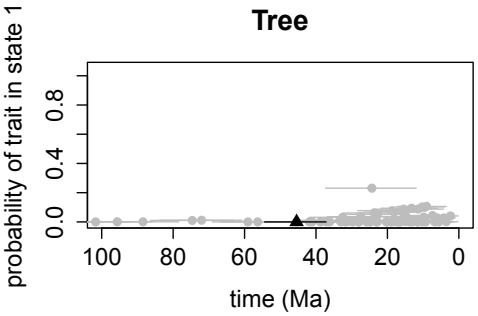
Ericaceae: Vaccinieae

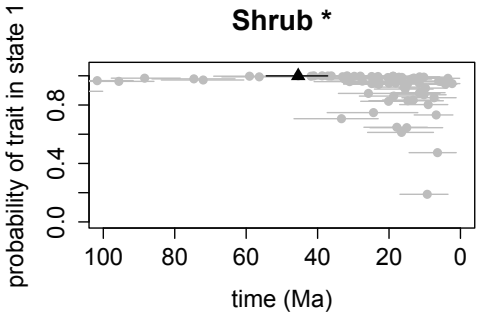


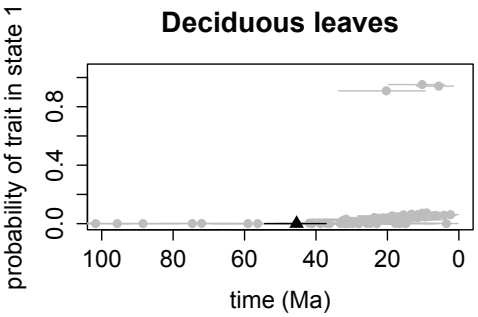




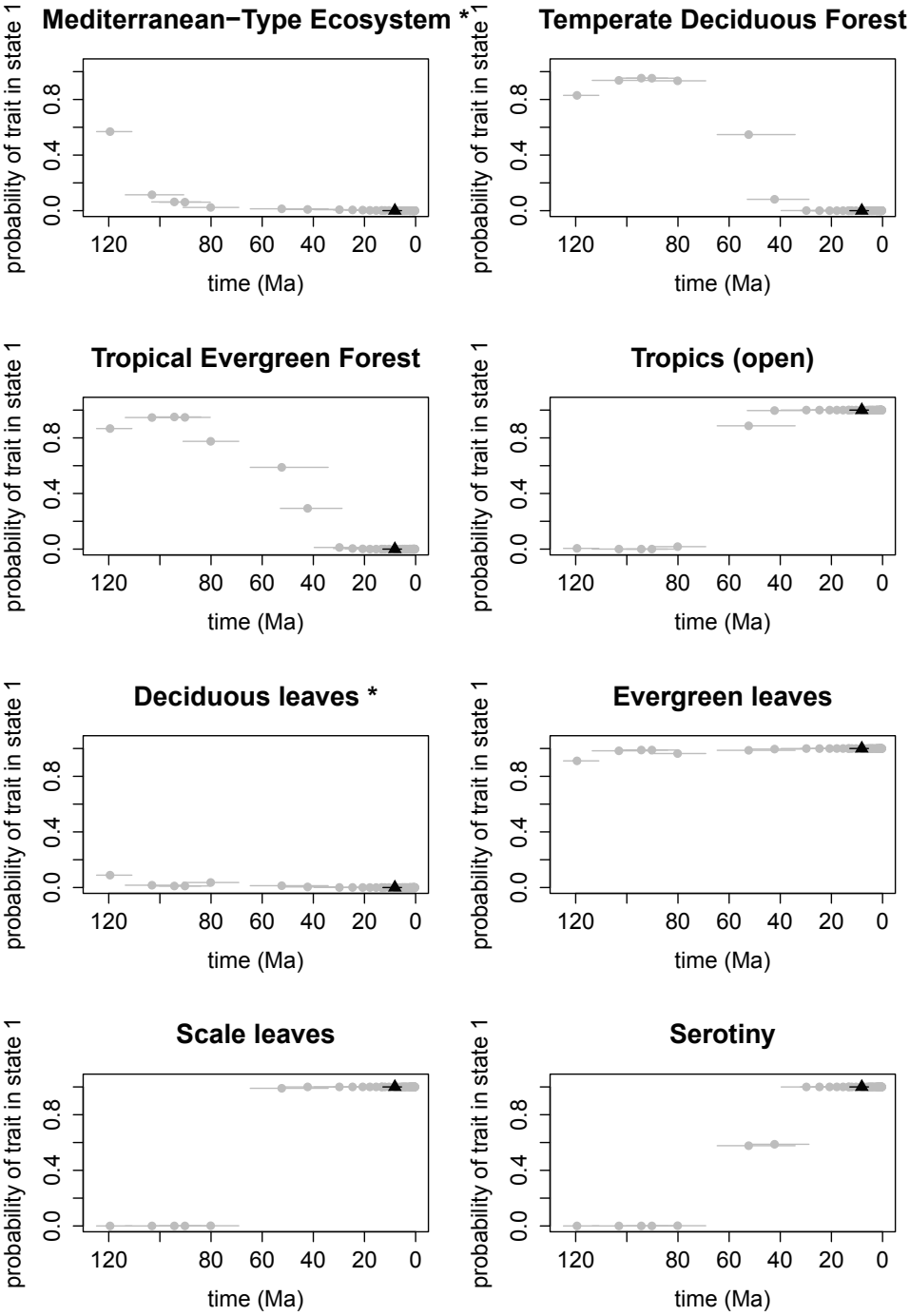




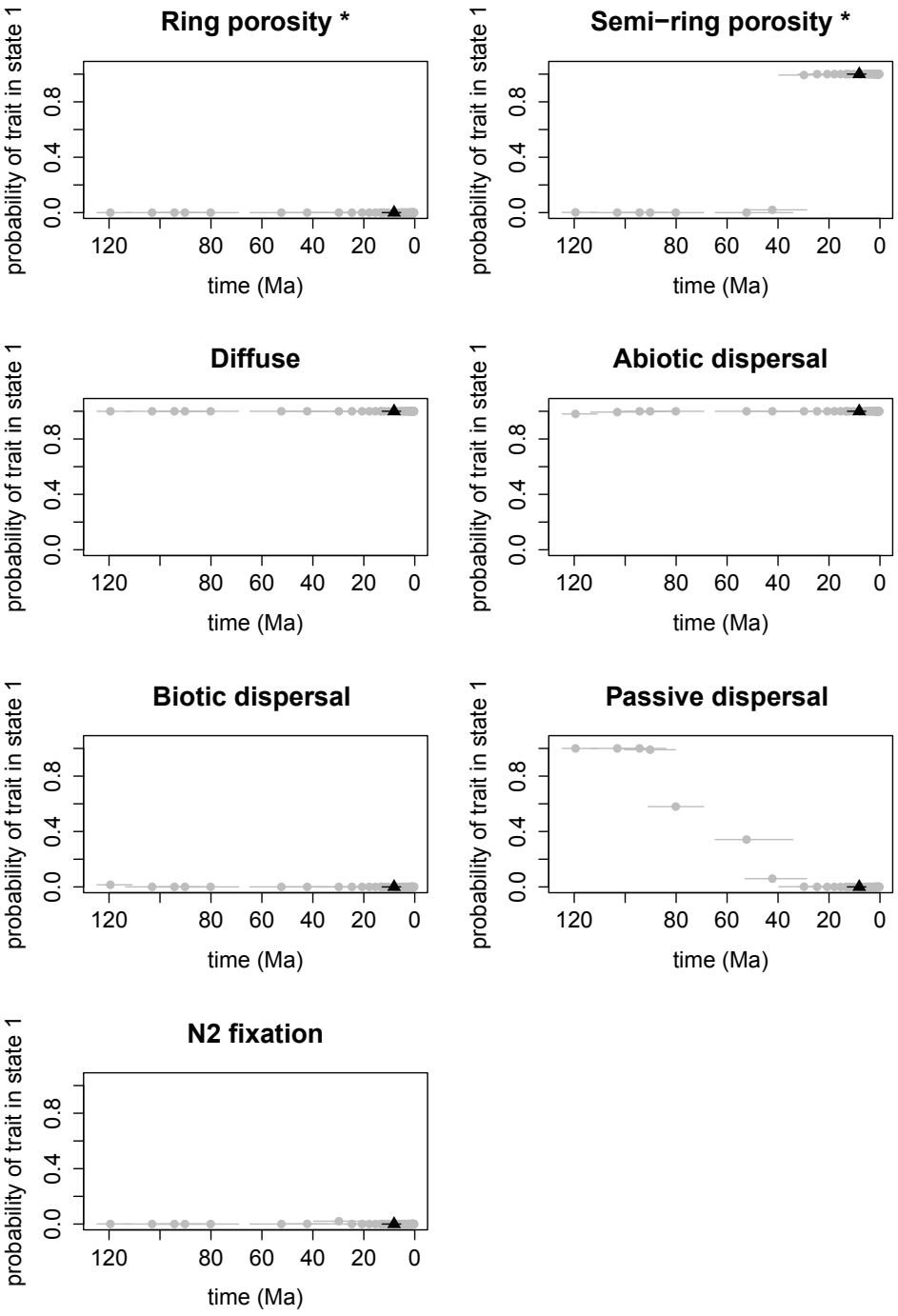




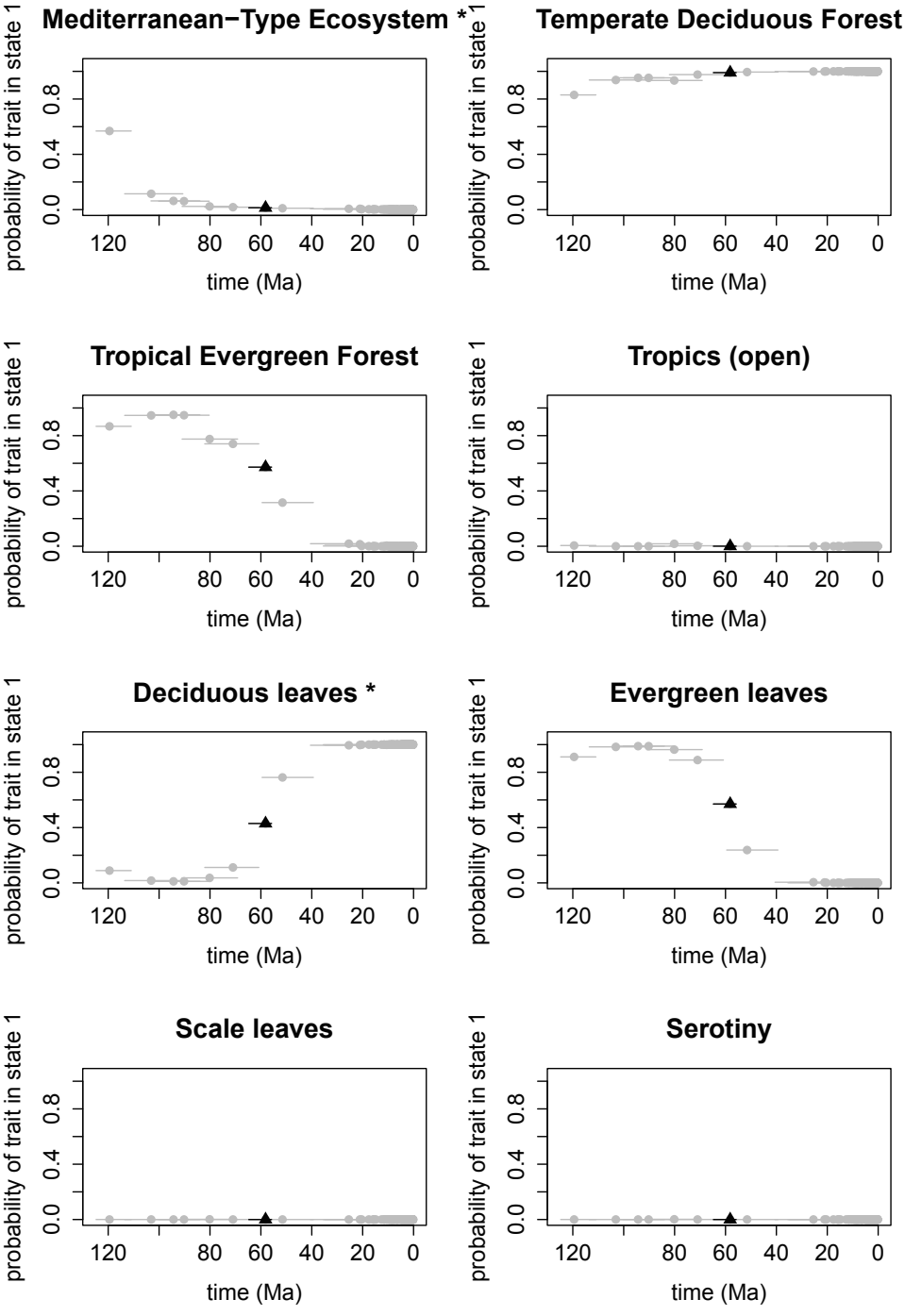
Fagales: *Allocasuarina* spp 1



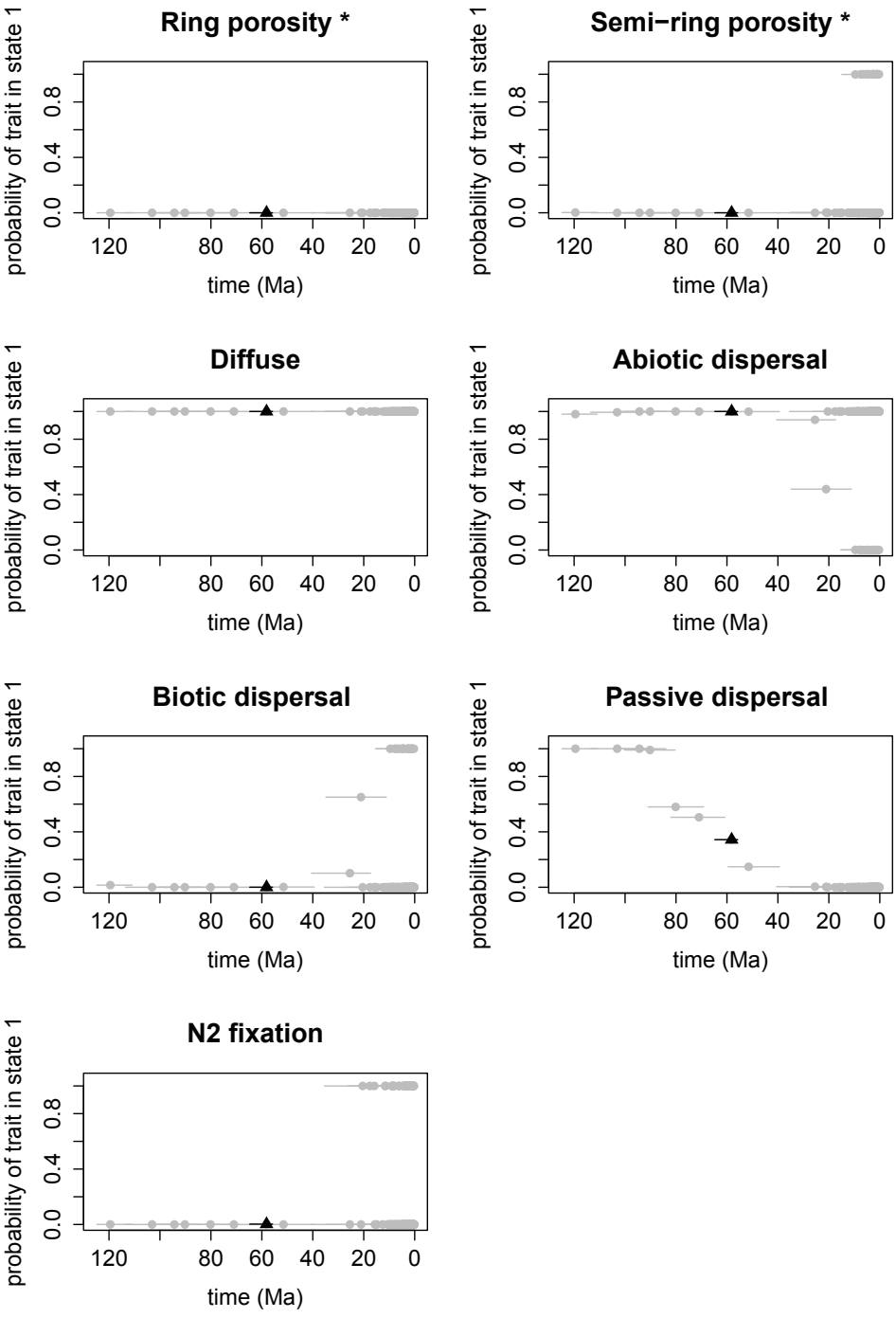
Fagales: *Allocasuarina* spp 2



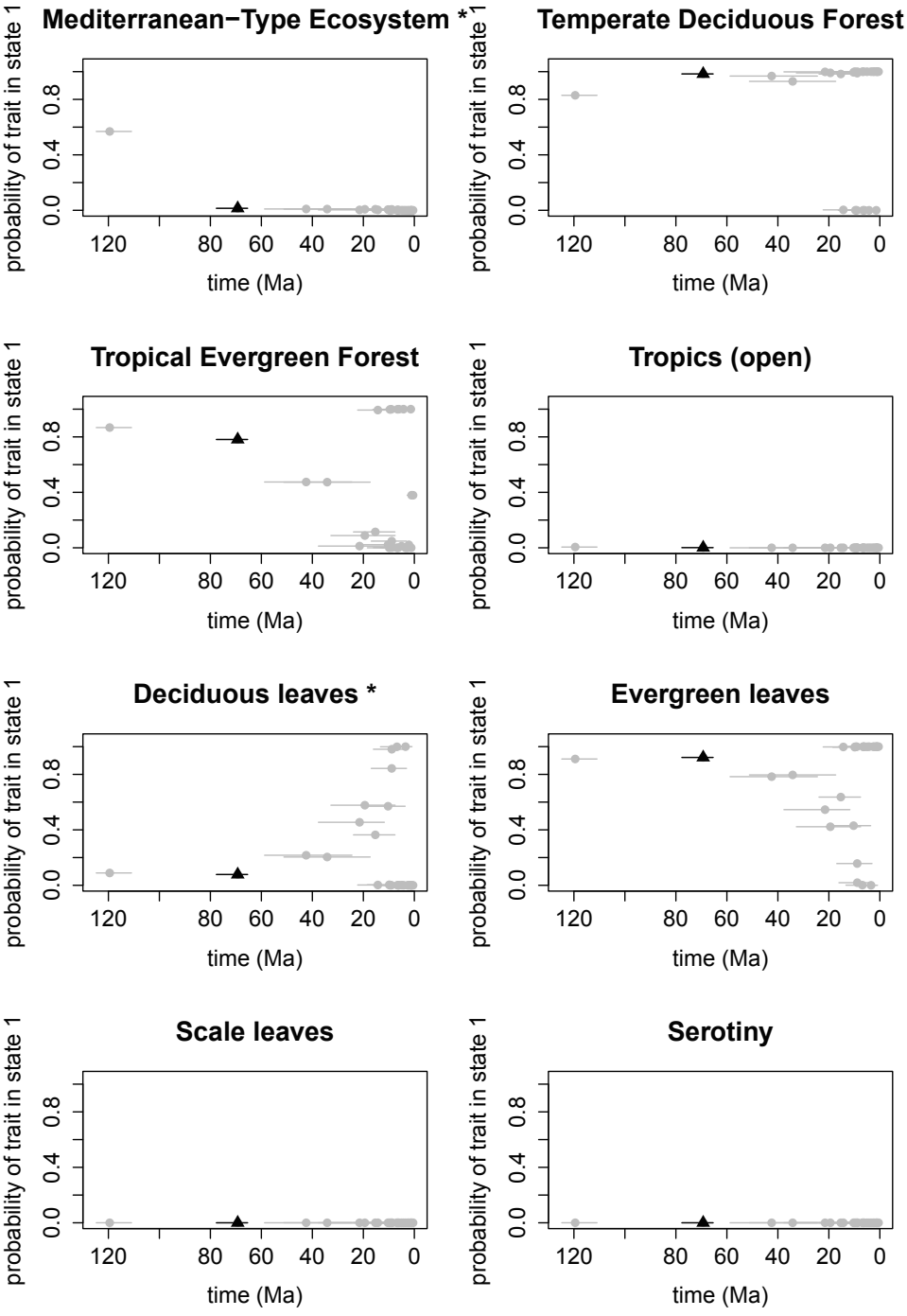
Fagales: Betulaceae spp 1



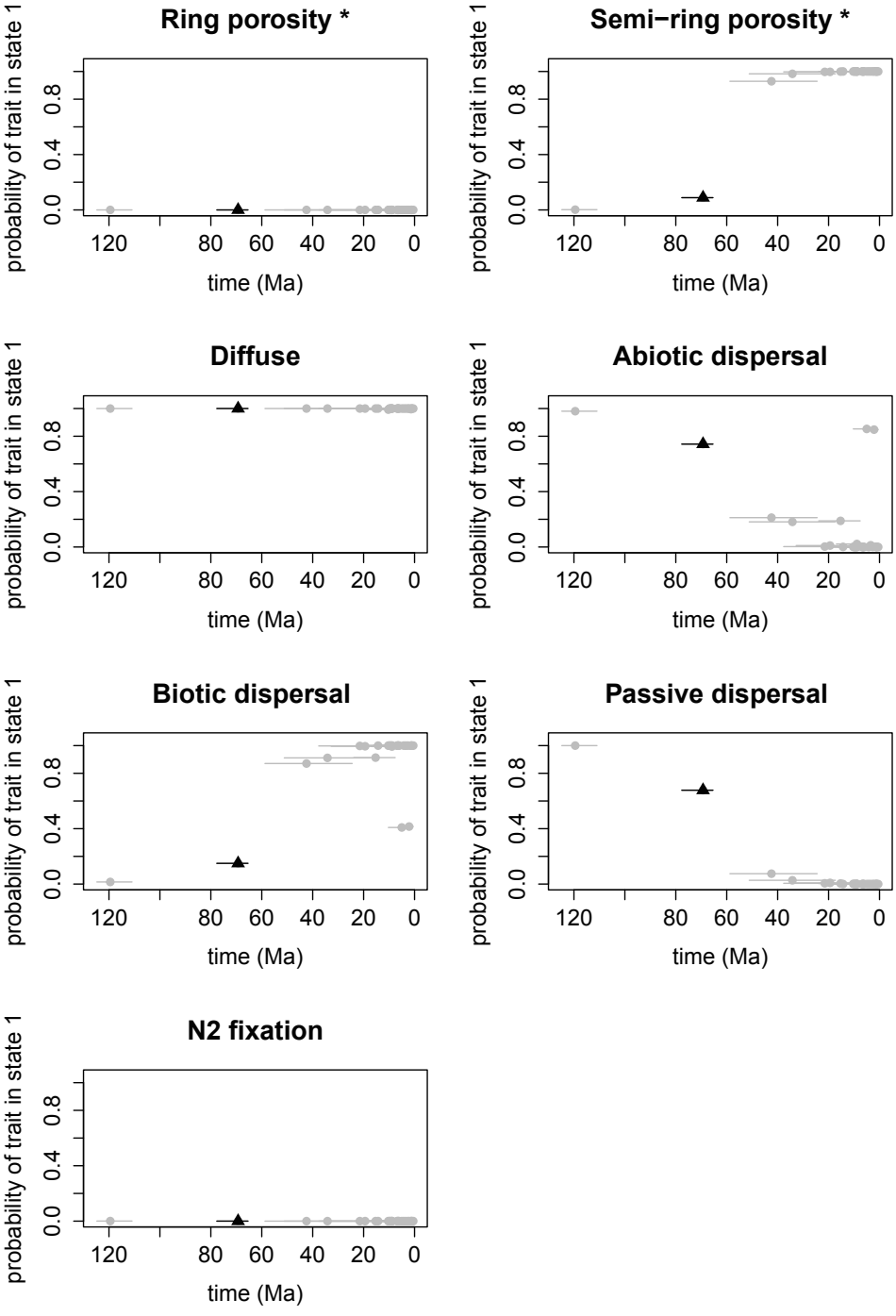
Fagales: Betulaceae spp 2



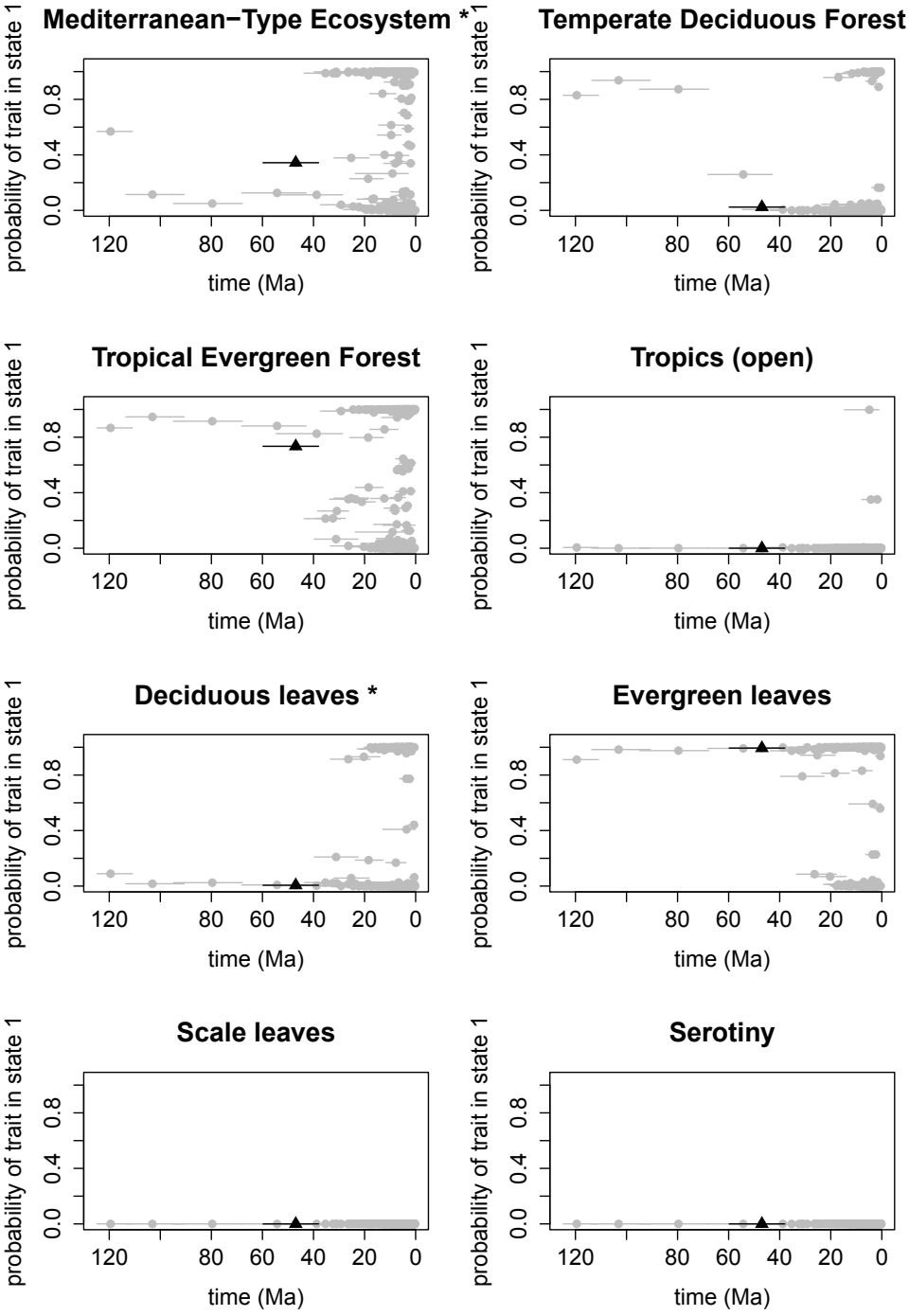
Fagales: *Nothofagus* 1



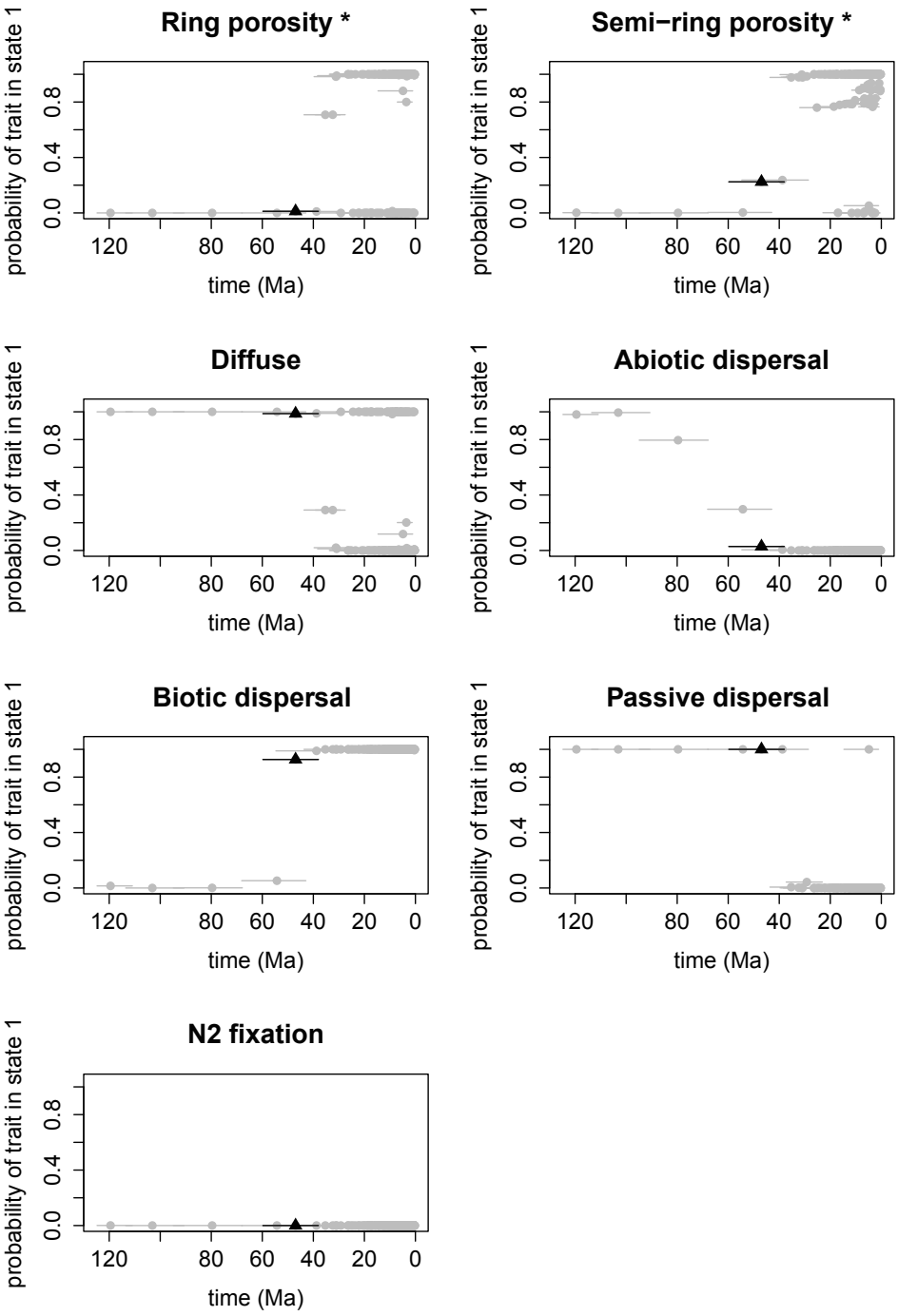
Fagales: *Nothofagus* 2



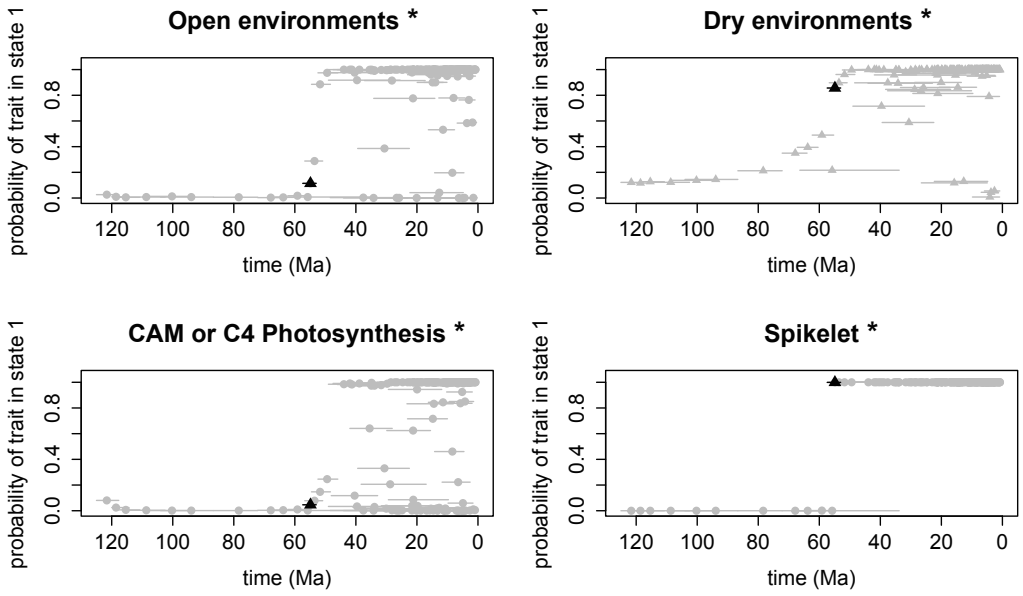
Fagales: quercoids 1



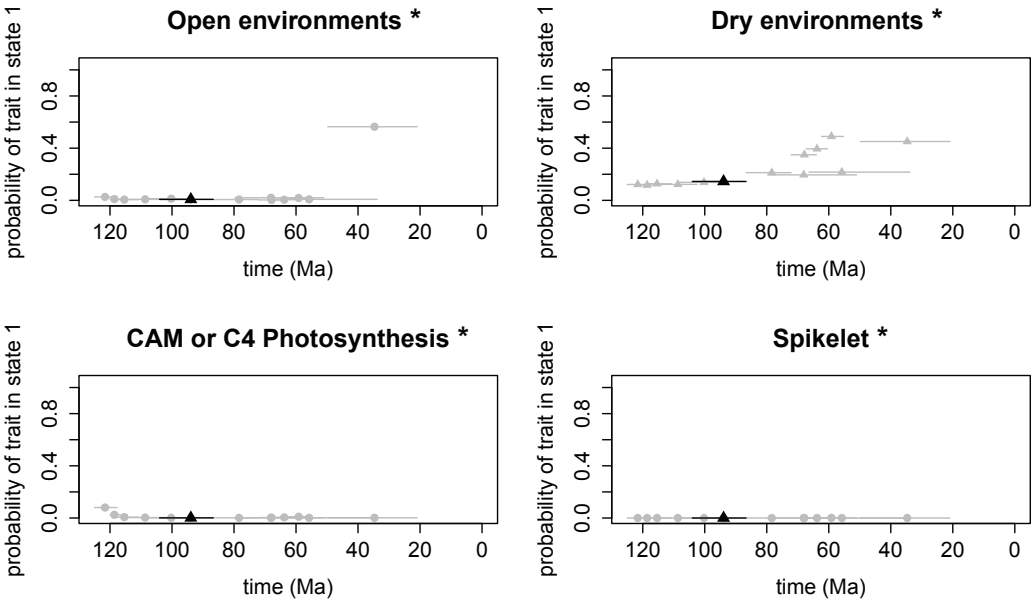
Fagales: quercoids 2



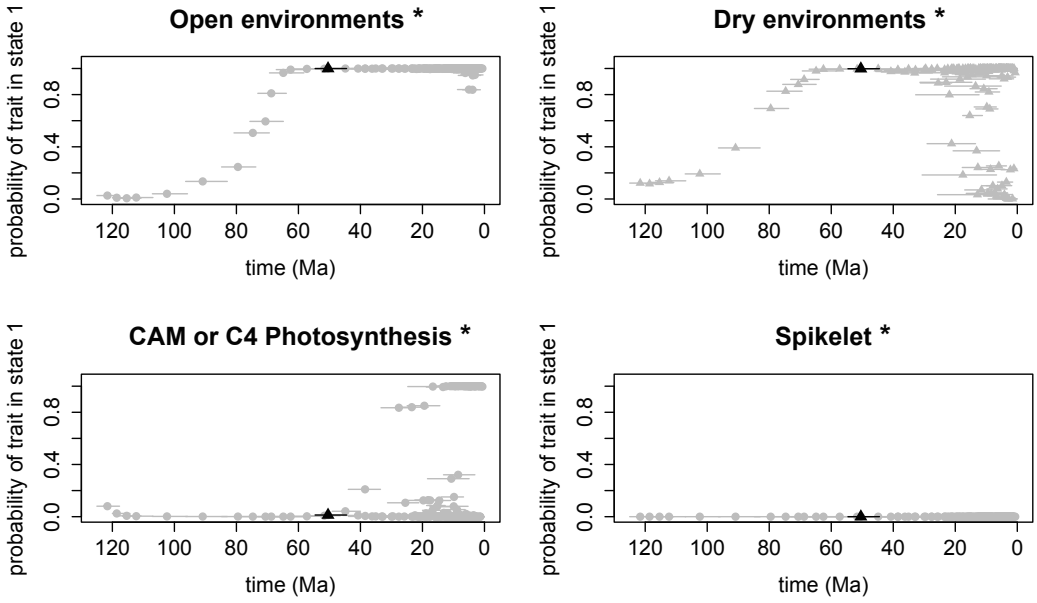
Poales: Poaceae



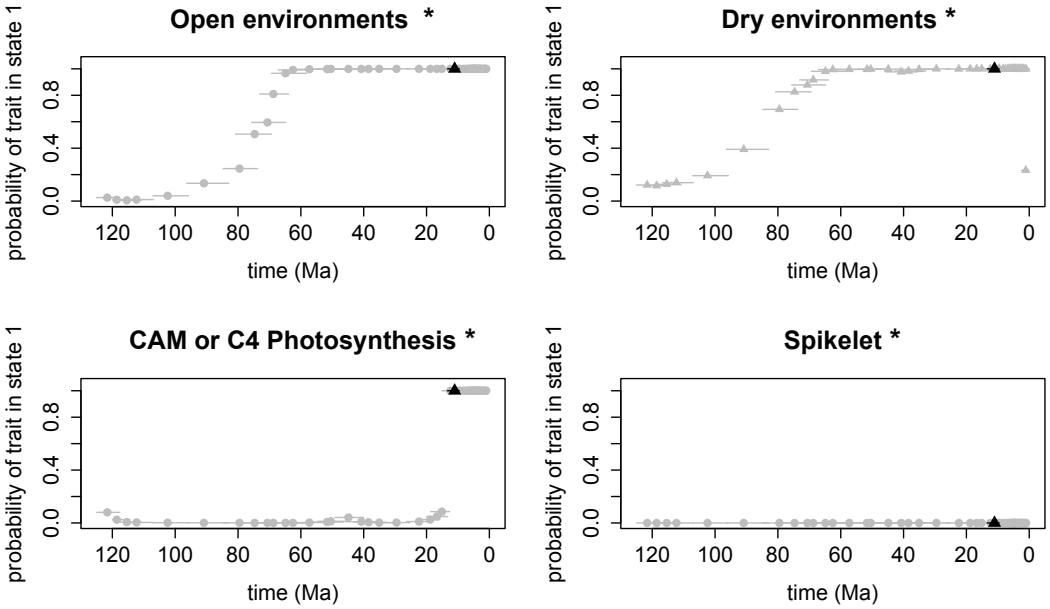
Poales: EDL graminids



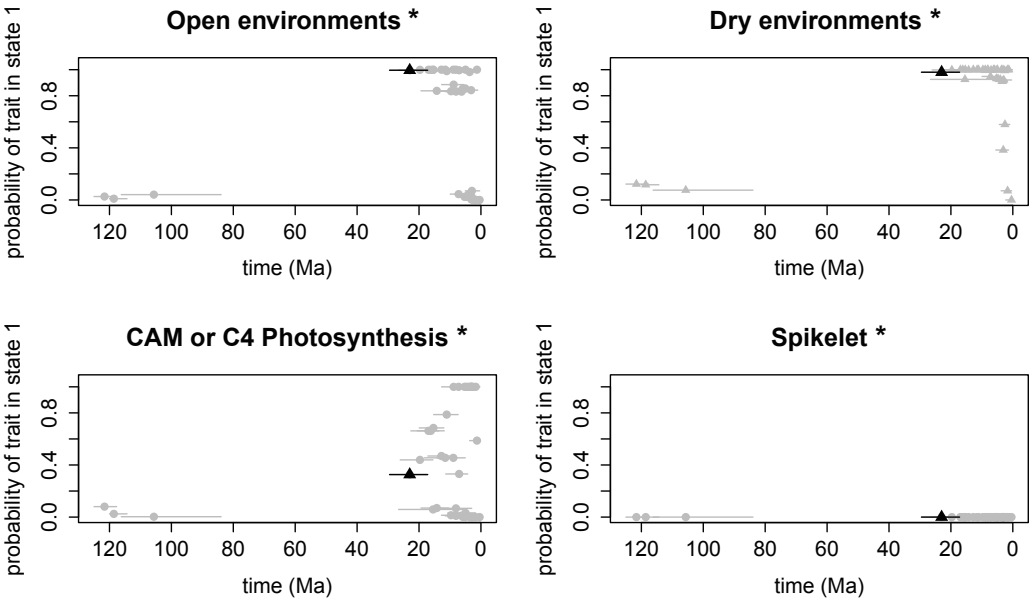
Poales: Cyperoideae



Poales: Cypereae



Poales: Bromeliaceae



CHAPTER VII: CONCLUDING REMARKS

The research in this thesis emphasizes the intricate interplay between biological diversity, morphological form, and the global environment. I have used phylogenetic comparative methods to investigate the dependence of diversification on trait innovation and on ecological strategies of lineages in a certain place at a certain time. In particular, this thesis demonstrates that:

(1) The interaction between vegetative traits and environments can influence diversification rates by evolving lineage-specific traits which allow survival under new environmental conditions (chapter II) or traits that reduce extinction rates in particular environments (chapter IV);
(2) Diversity in Mediterranean-type ecosystems (MTEs) may have resulted from habitat-dependent (Penaeaceae, Phyliceae, Diosmeae, chapter II) and MTE-dependent (Rhamnaceae, chapter III) diversification (i.e. speciation and extinction) rates;
(3) Sclerophyllous traits in Rhamnaceae may have been adaptive to nutrient-poor soils in Cape and Australian MTEs, and thus evolved as exaptations to the Mediterranean-climate (chapter IV);
(4) Climatic niche evolution and leaf trait disparification are coupled during radiation of the Proteaceae, and seem particularly fast in clades occurring in non-climatically buffered open environments, such as MTEs (chapter V);
(5) Intrinsic and extrinsic variables involved in radiating clades can be classified as backgrounds, triggers and modulators, and as being simple (conserved) or polymorphic (labile) during radiation (chapter VI).

Several points that were not or only briefly raised during the discussions of the individual chapters will be presented here to highlight emerging, overarching questions, identify major shortcomings in our knowledge of the role of functional traits in Cenozoic angiosperm radiations, and implications for the general view of ecological and evolutionary processes in creating global diversity patterns.

Ecological consequences of functional trait evolution during radiation

The occurrence of *in-situ* radiations in certain areas, and the role of functional traits in these radiations, may have important consequences for the assembly of ecological traits in ecological communities, and consequently 'functional diversity' (Emerson and Gillespie 2008). Functional diversity is "the value and range of functional traits of the organisms present in a given ecosystem" and directly affects ecosystem functioning, i.e. the "flow of energy and materials through the arrangement of biotic and abiotic components of an ecosystem" (Díaz and Cabido 2001). Functional diversity may thus depend on biological (taxonomic) diversity and functional trait evolution resulting from radiations.

Two aspects of radiating clades with respect to functional diversity are of importance. First, the degree of niche and trait conservatism during radiation may influence the functional diversity of the species-pool from which communities are assembled (Webb et al. 2002). Second, traits influencing diversification rates may create a species-pool dominated by certain functional traits and types. In chapter II we show that discrete shifts in leaf functional traits underlie the radiation in Cape fynbos clades from afromontane forest ancestors, at least in Penaeaceae and Phyliceae. Within the fynbos radiations, lineages show strong trait conservatism of small leaves with a low Specific leaf area (SLA), indicative of sclerophylly. This may lead to a species-pool dominated by species with

small low SLA leaves. In chapter IV we demonstrate that these sclerophyllous traits may influence diversification rates of lineages by reducing extinction rates in MTEs in the Cape and Australia. We argue that this may result in a flora dominated by species with sclerophyllous traits. These historical aspects may consequently influence, to some degree, the assembly of ecological communities.

Species enter and persist in local communities because of their ecological fit to local conditions, and these communities can be phylogenetically 'clustered' (species in communities are more similar compared to random assembly from the species-pool) or overdispersed (species in communities are less similar compared to random assembly from the species-pool) (Webb et al. 2002). The field of 'community phylogenetics' aims to connect these observations to the ecological processes of 'habitat filtering' and 'competitive exclusion', depending on whether traits are phylogenetically conserved or convergent (Webb et al. 2002, but see Losos 2008 for a critical review on phylogenetic conservatism). The results in this thesis suggest that the connection between pattern (e.g. phylogenetic clustering) and process (e.g. habitat filtering) in community phylogenetics may be partly disconnected, for at least two reasons. First, one of the assumptions in community phylogenetics is that traits evolve under Brownian motion and that closely related species are ecologically similar. We demonstrate that this can be wrong. For example, in chapters IV and V we establish that traits often do not follow a simple random model of Brownian motion, but instead complex modification of the Ornstein-Uhlenbeck model of trait evolution fit the data best. Furthermore, in chapter V we observe shifts in phenotypic rate regimes even within a single clade (Proteaceae). In addition, the presence of a correlation between the evolution of a trait and diversification rates can bias ancestral state reconstructions if models do not account for such dependency, and may thus additionally affect the selection of models best describing the evolution of the trait (Maddison 2006). Second, the sampling of communities from a species-pool assumes that the species-pool is a random sample of species, whereas, as indicated above, the species-pool may be biased by trait-dependent diversification or trait convergence / divergence.

Although the importance of diversification in species-richness patterns across biomes has been acknowledged for some time now (e.g. Pennington et al. 2006, Ricklefs 2006), I thus suggest that the complexity of trait evolution in a clade – i.e. leading to trait divergence and convergence under models other than Brownian motion – and the effects of traits on diversification rates may in addition affect the functional diversity of biomes, ecosystems and regional species-pools. I suggest that the inclusion of more complex models of trait evolution and the effect of traits on diversification rates in studies using community phylogenetics could enhance our understanding of spatial patterns of functional diversity.

How functional are functional traits?

One of the shortcomings in this thesis is the use of – often – single traits or multidimensional trait axes as proxies for ecological strategies, but these shortcomings nevertheless provide exciting possibilities for forthcoming research. The traits in this thesis were chosen to reflect plant strategies, such as those typical for species occurring in Mediterranean-type ecosystems. For example, in chapter II we used leaf area and SLA as proxies for the afromontane forest- *versus* fynbos-strategy and in chapter IV we assembled several binary traits and leaf area and SLA to determine non-sclerophyllous and sclerophyllous strategies. In chapter V we included leaf shape and leaf complexity as indicator traits of open and closed vegetation strategies, and in chapter VI the selection of traits was dependent on the taxonomic group (Ericaceae, Fagales and Poales) and environments encountered by these groups (e.g. mountains in Ericacecae, temperate forests in Fagales and open / shady habitats in grasses). Generally, the functionality of the selected traits was inferred based on the literature. This raises two questions. First, how 'functional' are the selected traits (what determines their association

with certain environments in terms of physiology and resource use)? And second, are they good proxies for our hypothesized functional strategies (i.e. how strong is the trait-environment match, and do they provide fitness benefits in these environments)?

Understanding how traits function (in terms of fitness consequences due to a trait-environment match) is essential to testing adaptation hypotheses, for example the hypothesis we state at the end of chapter IV: did the sclerophyllous trait syndrome evolve as an adaptation to nutrient-poor soils? A first approach to answering this question could be by measuring trait plasticity of our species under low and high nutrient conditions to test if variation in the selected traits indeed correlates with the environment for which we hypothesized its functionality. However, within-species plasticity does not necessarily relate to between-species adaptations. Next, we could therefore measure fitness components of (sister-) species which differ in their functional traits as well as their preference for soil nutrient levels before and after transplanting them to the non-preferred soil condition, to evaluate if this preference, and the traits correlated to this preference, may have evolved adaptively.

The choice of leaf area and SLA in this thesis is based on the easy and inexpensive measureable character of these 'soft' traits (Cornelissen et al. 2003), suitable to the large-scale and deep-time hypotheses tested in this thesis, and indicator traits of leaf economics (Wright et al. 2004). Furthermore, these traits are indicative of sclerophylly to some degree. Nevertheless, other 'hard' traits could possibly provide a more precise indication of sclerophylly and its response to certain environmental conditions. For example, there is a difference in traits related to low nutrient levels, low phosphorus and open habitats with high solar radiation (Jordan et al. 2005), called scleromorphic traits, and traits related to low water levels, called xeromorphic traits (Hill 1998). Scleromorpy has been associated with a small, evergreen, perennial habit, with an extensive root system (a large root: shoot ratio), and small, highly fibrous leaves (i.e. low SLA). Some of these features could also be regarded as adaptations to low water availability (e.g. small leaves, evergreen, low SLA), but an additional set of features could be unequivocally associated with low water availability, i.e. traits that reduce water loss such as presence of stomata in pits, stomata individually enclosed by raised structures or revolute leaf margins (Hill 1998). Based on fossil evidence, scleromorphy was observed early in the evolution of Proteaceae, whereas xeromorphy did not evolve prior to the Late Eocene (Hill 1998). Furthermore, present-day centres of diversity of scleromorphic Proteaceae have very nutrient poor soils, but include both wet and dry environments (Johnson and Briggs 1975). The differentiation between scleromorphic and xeromorphic traits could therefore provide an interesting angle to the evolution and adaptation of traits in MTEs, such as those for Rhamnaceae in chapter IV. Furthermore, the evolution of other traits, such as leaf dry matter content (construction costs, nutrient retention), specific force to punch (leaf strength, persistence) and leaf chlorophyll content (light uptake efficiency), may provide interesting insights in other aspect of plant performance during radiation.

Generality of our study with respect to angiosperm diversification

The results in this thesis provide general insights with respect to the role of vegetative functional traits in angiosperm diversification during a climatically dynamic period: the Cenozoic. I demonstrate that in several independent lineages, the evolution of certain traits or trait syndromes is correlated to increased rates of net diversification, and that this is conditional on the environment. This is illustrated by the correlation between shifts in functional traits, habitat shifts and diversification rate shifts (chapter II); habitat-dependent speciation and extinction rates (chapter III); trait-dependent diversification but only in interaction with certain habitats (chapter IV); the correlation between niche evolution and trait disparification during diversification (chapter V); and the mixture of indirect and

direct effects of traits and environments on diversification rate shifts (chapter VI). This emphasizes the importance of considering both intrinsic and extrinsic traits when studying angiosperm radiation, as was already suggested by Simpson in 1953 in his influential book "*The major features of evolution*" (Simpson 1953). Furthermore, the frequency of innovation, or gradual change of functional traits during lineage diversification and over time, even within a single taxonomic family, suggests that the 'reinvention' theory by Crepet and Niklas (2009) as explanation for the success of angiosperms could bear some truth. The direct dependence of the plant's vegetative morphology on the external abiotic environment suggests that these traits are required to be 'evolvable' when climate change is rapid and selection acts on survival (persistence) of lineages. These traits may therefore contribute to low extinction rates over macro-evolutionary time, for example in topographically and climatically-stable environments such as the Cape and Australian MTEs (chapter IV). This hypothesis needs further testing from different taxonomic groups, different environments and based on fossil data in addition to molecular data (due to the issues with disentangling speciation and extinction rates from molecular data, see Rabosky 2010, Xing et al. 2014, Beaulieu and O'Meara 2015). The success of angiosperms may thus be explained by the 'flexibility' of trait innovation without reaching evolutionary dead-ends, rather than by repeated innovation by itself.

Further insights into the genetic mechanisms leading to innovation, and detection of the underlying genes responsible for certain traits (e.g. precursor traits, Marazzi et al. 2012) could provide a better understanding of the proximate causes of trait innovation and the consequences on a micro-evolutionary scale. How often do traits evolve and become fixed in populations? What is the relative contribution of genetic change (mutation) and external selection on trait fixation? Can the interaction between vegetative traits and environments cause 'ecological speciation'? And how do these micro-evolutionary processes relate to the macro-evolutionary processes studied in this thesis? These, among the questions raised earlier in this thesis, could possibly form a basis for future research in plant systematics.

In conclusion, the results in this thesis show, based on several angiosperm clades, that angiosperm species richness may generally have resulted from many distinct, discrete radiations, each driven by a unique combination of traits and triggers. The importance of the interaction between (vegetative) traits and environments in angiosperm diversification was suggested previously (Simpson 1953, Davies and Barraclough 2006), as was the idea of repeated innovations (Crepet and Niklas 2009). I add to these ideas by concluding that the success of angiosperms as a result of radiations may be explained by their 'trait flexibility', i.e. the underlying, (possibly genetic) ability to evolve - gradually or punctually – functional traits, repeatedly over time, space and taxonomic clades.

LITERATURE CITED

Ackerly, D. D. 2004a. Adaptation, niche conservatism, and convergence: comparative studies of leaf evolution in the California chaparral. Am. Nat. **163**:654-671.

Ackerly, D. D. 2004b. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. Ecol. Monogr. **74**:25-44.

Ackerly, D. D. 2009. Evolution, origin and age of lineages in the Californian and Mediterranean floras. Journal of Biogeography **36**:1221-1233.

Adrain, J. M. and S. R. Westrop. 2003. Paleobiodiversity: we need new data. Paleobiology **29**:22-25.

Ake-Assi, L. 1963. Contribution à l'étude floristique de la Côte d'Ivoire. Encyclopedie Biologique, Paris.

Alfaro, M. E., F. Santini, C. Brock, H. Alamillo, A. Dornburg, D. L. Rabosky, G. Carnevale, and L. J. Harmon. 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proc. Natl. Acad. Sci. U.S.A. **106**:13410-13414.

Anacker, B. L., J. B. Whittall, E. E. Goldberg, and S. P. Harrison. 2011. Origins and consequences of serpentine endemism in the California flora. Evolution **65**:365-376.

Antonelli, A. and I. Sanmartin. 2011. Why are there so many plant species in the Neotropics? Taxon **60**:403-414.

Arakaki, M., P.-A. Christin, R. Nyffeler, A. Lendel, U. Eggli, R. M. Ogburn, E. Spriggs, M. J. Moore, and E. J. Edwards. 2011. Contemporaneous and recent radiations of the world's major succulent plant lineages. Proc. Natl. Acad. Sci. U.S.A. **108**:8379-8384.

Armesto, J. J., M. T. K. Arroyo, and L. F. Hinojosa. 2007. The Mediterranean environment of central Chile. Pages 184-199 *in* T. T. Veblen, K. R. Young, and A. R. Orme, editors. The physical geography of South America. Oxford University Press, Oxford.

Aschmann, H. 1973. Distribution and Peculiarity of Mediterranean Ecosystems. Pages 11-19 *in* F. Castri and H. A. Mooney, editors. Mediterranean Type Ecosystems: Origin and Structure. Springer Berlin Heidelberg.

Askin, R. A. and A. M. Baldoni. 1998. The santonian through paleogene record of Proteaceae in the southern South America – Antarctic peninsula region. Australian Systematic Botany **11**:373-390.

Axelrod, D. 1973. History of the Mediterranean Ecosystem in California. Pages 225-277 *in* F. Castri and H. A. Mooney, editors. Mediterranean Type Ecosystems: Origin and Structure. Springer Berlin Heidelberg.

Axelrod, D. I. 1985. Miocene Floras from the Middlegate Basin, West-Central Nevada. University of California Press, Berkeley.

Axelrod, D. I. 1995. The Miocene Purple Mountain flora of western Nevada. University of California Publications in Geological, Sciences, 139.

Baldwin, B. G. and M. J. Sanderson. 1998. Age and rate of diversification of the Hawaiian silversword alliance (Compositae). Proc. Natl. Acad. Sci. U.S.A. **95**:9402-9406.

Barker, N. P., P. H. Weston, F. Rutschmann, and H. Sauquet. 2007. Molecular dating of the 'Gondwanan' plant family Proteaceae is only partially congruent with the timing of the break-up of Gondwana. Journal of Biogeography **34**:2012-2027.

Barker, W., L. Haegi, and R. Barker. 1999. Hakea. Pages 1-170 *in* A. Wilson, editor. Flora of Australia. CSIRO Publishing, Melbourne.

Barnosky, A. D. 2001. Distinguishing the dffects of the Red Queen and Court Jester on Miocene mammal evolution in the Northern Rocky Mountains. Journal of Vertebrate Paleontology **21**:172-185.

Basinger, J. F. and D. L. Dilcher. 1984. Ancient bisexual flowers. Science **224**:511-513.

Beaulieu, J. M., D.-C. Jhwueng, C. Boettiger, and B. C. O'Meara. 2012. Modeling stabilizing selection: expanding the Ornstein-Uhlenbeck model of adaptive evolution. Evolution **66**:2369-2383.

Beaulieu, J. M. and B. C. O'Meara. 2015. Extinction can be estimated from moderately sized molecular phylogenies. Evolution:n/a-n/a.
Beerling, D. J. and C. P. Osborne. 2006. The origin of the savanna biome. Global Ecol. Biogeogr. **12**:2023-2031.
Belcher, C. M., L. Mander, G. Rein, F. X. Jervis, M. Haworth, S. P. Hesselbo, I. J. Glasspool, and J. C. McElwain. 2010. Increased fire activity at the Triassic/Jurassic boundary in Greenland due to climate-driven floral change. Nature Geosci. **3**:426-429.
Bell, C. D., E. V. Mavrodiev, P. S. Soltis, A. K. Calaminus, D. C. Albach, N. Cellinese, N. Garcia-Jacas, and D. E. Soltis. 2012. Rapid diversification of Tragopogon and ecological associates in Eurasia. J. Evol. Biol. **25**:2470-2480.
Bell, C. D. and R. W. Patterson. 2000. Molecular Phylogeny and Biogeography of Linanthus (Polemoniaceae). Am. J. Bot. **87**:1857-1870.
Bell, C. D., D. E. Soltis, and P. S. Soltis. 2010. The age and diversification of the angiosperms re-revisited. Am. J. Bot. **97**:1296-1303.
Benton, M. J. 2009. The Red Queen and the Court Jester: Species diversity and the role of biotic and abiotic factors through time. Science **323**:728-732.
Benzing, D. H. 1980. The biology of Bromeliads. CA: Eureka Printing Company, Inc.
Bew, J. 1929. The World's grasses.Their differentiation, distribution, economics and ecology. Longmans, Green and Co., London.
Bond, W., G. Midgley, and F. Woodward. 2003. What controls South African vegetation-climate or fire? South African Journal of Botany **69**:79-91.
Bond, W. J., F. I. Woodward, and G. F. Midgley. 2005. The global distribution of ecosystems in a world without fire. New Phytologist **165**:525-538.
Bouchenak-Khelladi, Y., A. M. Muasya, and H. P. Linder. 2014. A revised evolutionary history of Poales: origins and diversification. Botanical Journal of the Linnean Society **175**:4-16.
Bouchenak-Khelladi, Y., R. E. Onstein, Y. Xing, O. Schwery, and H. P. Linder. 2015. On the complexity of triggering evolutionary radiations. New. Phytol. doi: 10.1111/nph.13331.
Boucher, F. C., W. Thuiller, C. Roquet, R. Douzet, S. Aubert, N. Alvarez, and S. Lavergne. 2012. Reconstructing the origins of high-alpine niches and cushion life form in the genus *Androsace* s.l. (Primulaceae). Evolution **66**:1255-1268.
Bradford, M., D. Metcalfe, A. Ford, M. Liddell, and A. McKeown. 2014. Floristics, stand structure and above ground 1 biomass of a 25 ha rainforest plot in the Wet Tropics of Australia. J. Trop. For. Sci. **26**:543-553.
Briggs, B. G. 2011. *Ecdeiocolea rigens*, a new species of Ecceiocoleaceae (Poales) from Western Australia. Telopea **13**:69-75.
Briggs, B. G., A. D. Marchant, and A. J. Perkins. 2014. Phylogeny of the restiid clade (Poales) and implications for the classification of Anarthriaceae, Centrolepidaceae and Australian Restionaceae. Taxon **63**:24-46.
Bromham, L., X. Hua, R. Lanfear, and P. F. Cowman. 2015. Exploring the relationships between mutation rates, life history, genome size, environment, and species richness in flowering plants. The American Naturalist **185**:507-524.
Buerki, S., S. Jose, S. R. Yadav, P. Goldblatt, J. C. Manning, and F. Forest. 2012. Contrasting Biogeographic and Diversification Patterns in Two Mediterranean-Type Ecosystems. PLoS ONE **7**:e39377.
Buerki, S., J. C. Manning, and F. Forest. 2013. Spatio-temporal history of the disjunct family Tecophilaeaceae: a tale involving the colonization of three Mediterranean-type ecosystems. Ann. Bot. **111**:361-373.
Burge, D. O., D. M. Erwin, M. B. Islam, J. Kellermann, S. W. Kembel, D. H. Wilken, and P. S. Manos. 2011. Diversification of Ceanothus (Rhamnaceae) in the California Floristic Province. Int. J. Plant Sci. **172**:1137-1164.
Burge, D. O. and S. R. Manchester. 2008. Fruit Morphology, Fossil History, and Biogeography of Paliurus (Rhamnaceae). Int. J. Plant Sci. **169**:1066-1085.
Bush, C. M., S. J. Wagstaff, P. W. Fritsch, and K. A. Kron. 2009. The phylogeny, biogeography and morphological evolution of *Gaultheria* (Ericaceae) from Australia and New Zealand. Australian Systematic Botany **22**:229-242.

Butler, M., A. and A. King, A. . 2004. Phylogenetic Comparative Analysis: A Modeling Approach for Adaptive Evolution. The American Naturalist **164**:683-695.
Byrne, M., D. A. Steane, L. Joseph, D. K. Yeates, G. J. Jordan, D. Crayn, K. Aplin, D. J. Cantrill, L. G. Cook, M. D. Crisp, J. S. Keogh, J. Melville, C. Moritz, N. Porch, J. M. K. Sniderman, P. Sunnucks, and P. H. Weston. 2011. Decline of a biome: evolution, contraction, fragmentation, extinction and invasion of the Australian mesic zone biota. Journal of Biogeography **38**:1635-1656.
Byrne, M., D. K. Yeates, L. Joseph, M. Kearney, J. Bowler, M. A. J. Williams, S. Cooper, S. C. Donnellan, J. S. Keogh, R. Leys, J. Melville, D. J. Murphy, N. Porch, and K. H. Wyrwoll. 2008. Birth of a biome: insights into the assembly and maintenance of the Australian arid zone biota. Molecular Ecology **17**:4398-4417.
Bytebier, B., A. Antonelli, D. U. Bellstedt, and H. P. Linder. 2010. Estimating the age of fire in the Cape flora of South Africa from an orchid phylogeny. Proc. R. Soc. Lond. B **278**:188-195.
Calvillo-Canadell, L. and S. R. S. Cevallos-Ferriz. 2007. Reproductive structures of Rhamnaceae from the Cerro del Pueblo (Late Cretaceous, Coahuila) and Coatzingo (Oligocene, Puebla) Formations, Mexico. Am. J. Bot. **94**:1658-1669.
Cardillo, M. 1999. Latitude and rates of diversification in birds and butterflies. Proc. R. Soc. Lond. B **266**:1221-1221.
Cardillo, M. and R. Pratt. 2013. Evolution of a hotspot genus: geographic variation in speciation and extinction rates in Banksia (Proteaceae). BMC Evol. Biol. **13**:155.
Carlson, J. E., K. E. Holsinger, and R. Prunier. 2011. Plant responses to climate in the Cape Floristic Region of South Africa: evidence for adaptive differentiation in the Proteaceae. Evolution **65**:108-124.
Carpenter, R. 2012. Proteaceae leaf fossils: phylogeny, diversity, ecology and Austral distributions. The Botanical Review **78**:261-287.
Carpenter, R. J. 1994. Cuticular morphology and aspects of the ecology and fossil history of north Queensland rainforest Proteaceae. Botanical Journal of the Linnean Society **116**:249-303.
Carpenter, R. J., J. M. Bannister, G. J. Jordan, and D. E. Lee. 2010. Leaf fossils of Proteaceae tribe Persoonieae from the Late Oligocene–Early Miocene of New Zealand. Australian Systematic Botany **23**:1-15.
Carpenter, R. J. and G. J. Jordan. 1997. Early Tertiary macrofossils of Proteaceae from Tasmania. Australian Systematic Botany **10**:533-563.
Castri, F. 1973. Climatographical Comparisons between Chile and the Western Coast of North America. Pages 21-36 *in* F. Castri and H. A. Mooney, editors. Mediterranean Type Ecosystems: Origin and Structure. Springer Berlin Heidelberg.
Castroviejo, S., C. Aedo, S. Cirujan, M. Lainz, P. Montserrat, R. Morales, F. Munoz Garmendia, C. Navarro, J. Paiva, and C. Soriano. 1993. Flora Iberica 4. Real Jardin Botanico. CSIC, Madri.
Chen, J., S. C. Saunders, T. R. Crow, R. J. Naiman, K. D. Brosofske, G. D. Mroz, B. L. Brookshire, and J. F. Franklin. 1999. Microclimate in forest ecosystem and landscape ecology: variations in local climate can be used to monitor and compare the effects of different management regimes. BioScience **49**:288-297.
Christin, P.-A., C. P. Osborne, D. S. Chatelet, J. T. Columbus, G. Besnard, T. R. Hodkinson, L. M. Garrison, M. S. Vorontsova, and E. J. Edwards. 2013. Anatomical enablers and the evolution of C4 photosynthesis in grasses. Proceedings of the National Academy of Sciences **110**:1381-1386.
Christophel, D. 1984. Early tertiary Proteaceae: The first floral evidence for the Musgraveinae. Australian Journal of Botany **32**:177-186.
Christophel, D. and D. Greenwood. 1987. A megafossil flora from the Eocene of Golden Grove, South Australia. Transactions of the Royal Society of South Australia **111**:155-162.
Clayton, W. D., M. S. Vorontsova, K. T. Harman, and H. Williamson. 2006. GrassBase-The Online World Grass Flora.
Clinton, B. D. 2003. Light, temperature, and soil moisture responses to elevation, evergreen understory, and small canopy gaps in the southern Appalachians. Forest Ecology and Management **186**:243-255.

Cody, M. L. and H. A. Mooney. 1978. Convergence versus nonconvergence in Mediterranean-climate ecosystems. Annual Review of Ecology, Evolution, and Systematics:265-321.
Coetzee, J. A. and J. Rogers. 1982. Palynological and lithological evidence for the miocene palaeoenvironment in the Saldanha region (South Africa). Palaeogeogr. Palaeoclimatol. Palaeoecol. **39**:71-85.
Cookson, I. 1950. Fossil pollen grains of proteaceous type from Tertiary deposits in Australia. Australian Journal of Scientific Research, Series B: Biological Sciences **3**:166-177.
Cornelissen, J. H. C., S. Lavorel, E. Garnier, S. Díaz, N. Buchmann, D. E. Gurvich, P. B. Reich, H. t. Steege, H. D. Morgan, M. G. A. v. d. Heijden, J. G. Pausas, and H. Poorter. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian Journal of Botany **51**:335-380.
Cornwell, W. K. and D. D. Ackerly. 2009. Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. Ecol. Monogr. **79**:109-126.
Cornwell, W. K., M. Westoby, D. S. Falster, R. G. FitzJohn, B. C. O'Meara, M. W. Pennell, D. J. McGlinn, J. M. Eastman, A. T. Moles, P. B. Reich, D. C. Tank, I. J. Wright, L. Aarssen, J. M. Beaulieu, R. M. Kooyman, M. R. Leishman, E. T. Miller, Ü. Niinemets, J. Oleksyn, A. Ordonez, D. L. Royer, S. A. Smith, P. F. Stevens, L. Warman, P. Wilf, and A. E. Zanne. 2014. Functional distinctiveness of major plant lineages. Journal of Ecology **102**:345-356.
Correa, E., C. Jaramillo, S. Manchester, and M. Gutierrez. 2010. A fruit and leaves of Rhamnaceous affinities from the late Cretaceous (Maastrichtian) of Colombia. Am. J. Bot. **97**:71-79.
Couper, R. A. 1953. Upper mesozoic and cainozoic spores and pollen grains from New Zealand. New Zealand Geological Survey Paleontological Bulletin **22**:77.
Cowling, R., F. Ojeda, B. Lamont, and P. Rundel. 2004. Climate stability in Mediterranean-type ecosystems: implications for the evolution and conservation of biodiversity.*in* Proceedings of the 10th MEDECOS—International Conference on Ecology, Conservation and Management of Mediterranean Climate Ecosystems, Rhodes Island, Greece, 25 April–1 May 2004.
Cowling, R. M. and P. M. Holmes. 1992. Flora and vegetation. Pages 23-61 *in* R. M. Cowling, editor. The Ecology of Fynbos. Nutrients, Fire and Diversity. Oxford University Press., Cape Town.
Cowling, R. M. and A. T. Lombard. 2002. Heterogeneity, speciation/extinction history and climate: explaining regional plant diversity patterns in the Cape Floristic Region. Divers. Distrib. **8**:163-179.
Cowling, R. M., F. Ojeda, B. B. Lamont, P. W. Rundel, and R. Lechmere-Oertel. 2005. Rainfall reliability, a neglected factor in explaining convergence and divergence of plant traits in fire-prone mediterranean-climate ecosystems. Global Ecol. Biogeogr. **14**:509-519.
Cowling, R. M., A. J. Potts, P. L. Bradshaw, J. Colville, M. Arianoutsou, S. Ferrier, F. Forest, N. M. Fyllas, S. D. Hopper, F. Ojeda, Ş. Procheş, R. J. Smith, P. W. Rundel, E. Vassilakis, and B. R. Zutta. 2014. Variation in plant diversity in mediterranean-climate ecosystems: the role of climatic and topographical stability. Journal of Biogeography:n/a-n/a.
Cowling, R. M., Ş. Procheş, and T. C. Partridge. 2009. Explaining the uniqueness of the Cape flora: Incorporating geomorphic evolution as a factor for explaining its diversification. Molecular Phylogenetics and Evolution **51**:64-74.
Cowling, R. M., P. W. Rundel, B. B. Lamont, M. Kalin Arroyo, and M. Arianoutsou. 1996. Plant diversity in mediterranean-climate regions. Trends Ecol. Evol. **11**:362-366.
Cowling, R. M. and E. T. F. Witkowski. 1994. Convergence and non-convergence of plant traits in climatically and edaphically matched sites in Mediterranean Australia and South Africa. Australian Journal of Ecology **19**:220-232.
Cox, B. 2001. The biogeographic regions reconsidered. J. Biogeogr. **28**:511-523.
Crayn, D. M., M. Rossetto, and D. J. Maynard. 2006. Molecular phylogeny and dating reveals an Oligo-Miocene radiation of dry-adapted shrubs (former Tremandraceae) from rainforest tree progenitors (Elaeocarpaceae) in Australia. Am. J. Bot. **93**:1328-1342.
Crepet, W. L. and K. J. Niklas. 2009. Darwin's second 'abominable mystery': Why are there so many angiosperm species? Am. J. Bot. **96**:366-381.
Crepet, W. L., K. C. Nixon, and M. A. Gandolfo. 2004. Fossil evidence and phylogeny: the age of major angiosperm clades based on mesofossil and macrofossil evidence from Cretaceous deposits. Am. J. Bot. **91**:1666-1682.

Crisp, M., L. Cook, and D. Steane. 2004. Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present–day communities? Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **359**:1551-1571.

Crisp, M. D., M. T. K. Arroyo, L. G. Cook, M. A. Gandolfo, G. J. Jordan, M. S. McGlone, P. H. Weston, M. Westoby, P. Wilf, and H. P. Linder. 2009. Phylogenetic biome conservatism on a global scale. Nature **458**:754-756.

Crisp, M. D. and L. G. Cook. 2009. Explosive radiation or cryptic mass extinction? Interpreting signatures in molecular phylogenies. Evolution **63**:2257-2265.

Crisp, M. D. and L. G. Cook. 2013. How was the Australian flora assembled over the last 65 million years? A molecular phylogenetic perspective. Annual Review of Ecology, Evolution, and Systematics **44**:303-324.

Dahlgren, R. M. T. and F. N. Rasmussen. 1983. Monocotyledon evolution: characters and phylogenetic estimation. . Evolutionary Biology **16**:255-395.

Davidian, H. H. 1992. The Rhododendron species. Timber Press, Portland.

Davies, T. and T. Barraclough. 2006. 10 The Diversification of Flowering Plants through Time and Space: Key Innovations, Climate and Chance. Pages 149–164 *in* T. Hodkinson, J. Parnell, and S. Waldren, editors. Reconstructing the tree of life: taxonomy and systematics of species rich taxa. Press, CRC.

Davis, M., P. Midford, and W. Maddison. 2013. Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. BMC Evol. Biol. **13**:38.

Deacon, H. J. 1983. The Comparative Evolution of Mediterranean-Type Ecosystems: A Southern Perspective. Pages 3-40 *in* F. J. Kruger, D. T. Mitchell, and J. U. M. Jarvis, editors. Mediterranean-Type Ecosystems: The Role of Nutrients. Springer Berlin Heidelberg.

Dettmann, M. and H. Clifford. 2005. Fossil fruit of the Grevilleeae (Proteaceae) in the Tertiary of eastern Australia. MEMOIRS-QUEENSLAND MUSEUM **51**:423.

Dettmann, M. E. and D. M. Jarzen. 1996. Pollen of proteaceous-type from latest Cretaceous sediments, southeastern Australia. Alcheringa: An Australasian Journal of Palaeontology **20**:103-160.

Dettmann, M. E. and D. M. Jarzen. 1998. The early history of the Proteaceae in Australia: the pollen record. Australian Systematic Botany **11**:401-438.

Díaz, S. and M. Cabido. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends in Ecology & Evolution **16**:646-655.

Diekmann, B., M. Fälker, and G. Kuhn. 2003. Environmental history of the south-eastern South Atlantic since the Middle Miocene: evidence from the sedimentological records of ODP Sites 1088 and 1092. Sedimentology **50**:511-529.

Dixon, P. and Dixon. 2003. VEGAN, a package of R functions for community ecology. Journal of Vegetation Science **14**:927-930.

Dobzhansky, T. 1950. Evolution in the tropics. American Scientist **38**:209-221.

Donoghue, M. J. and M. J. Sanderson. 2015. Confluence, synnovation, and depauperons in plant diversification. New Phytologist doi: 10.1111/nph.13367.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem bull **19**:11-15.

Drummond, A. and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology **7**:214.

Drummond, A. J., S. Y. W. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. **4**:e88.

Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012a. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution **29**:1969-1973.

Drummond, C. S., R. J. Eastwood, S. T. S. Miotto, and C. E. Hughes. 2012b. Multiple continental radiations and correlates of diversification in *Lupinus* (Leguminosae): testing for key innovation with incomplete taxon sampling. Syst. Biol. **61**:443-460.

Dupont, L. M., H. P. Linder, F. Rommerskirchen, and E. Schefuß. 2011. Climate-driven rampant speciation of the Cape flora. Journal of Biogeography **38**:1059-1068.

Dynesius, M. and R. Jansson. 2000. Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. Proc. Natl. Acad. Sci. U.S.A. **97**:9115-9120.
Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research **32**:1792-1797.
Edwards, E. J. and S. A. Smith. 2010. Phylogenetic analyses reveal the shady history of C4 grasses. Proceedings of the National Academy of Sciences of the United States of America **107**:2532-2537.
Eldrett, J. S., D. R. Greenwood, I. C. Harding, and M. Huber. 2009. Increased seasonality through the Eocene to Oligocene transition in northern high latitudes. Nature **459**:969-973.
Ellis, B., D. Daly, L.J. Hickey, K.R. Johnson, J. Mitchell, P. Wilf, and S. L. Wing. 2009. Manual of leaf architecture. Cornell Univ. Press, Ithaka.
Emerson, B. C. and R. G. Gillespie. 2008. Phylogenetic analysis of community assembly and structure over space and time. Trends in Ecology & Evolution **23**:619-630.
Etienne, R. S., B. Haegeman, T. Stadler, T. Aze, P. N. Pearson, A. Purvis, and A. B. Phillimore. 2011. Diversity-dependence brings molecular phylogenies closer to agreement with the fossil record. Proc. R. Soc. B.
Evans, Margaret E. K., Stephen A. Smith, Rachel S. Flynn, and Michael J. Donoghue. 2009. Climate, Niche Evolution, and Diversification of the "Bird-Cage" Evening Primroses (Oenothera, Sections Anogra and Kleinia). Am. Nat. **173**:225-240.
Ezard, T. H. G., T. Aze, P. N. Pearson, and A. Purvis. 2011. Interplay Between Changing Climate and Species' Ecology Drives Macroevolutionary Dynamics. Science **332**:349-351.
Farmer, G. T. and J. Cook. 2013. Pleistocene Glaciations. Pages 407-427 Climate Change Science: A Modern Synthesis. Springer Netherlands.
Feild, T. S., N. C. Arens, J. A. Doyle, T. E. Dawson, and M. J. Donoghue. 2004. Dark and disturbed: a new image of early angiosperm ecology. Paleobiology **30**:82-107.
FitzJohn, R., W. Maddison, and S. Otto. 2009. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. **58**:595 - 611.
FitzJohn, R. G. 2010. Quantitative traits and diversification. Systematic Biology **59**:619-633.
FitzJohn, R. G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods in Ecology and Evolution **3**:1084-1092.
Fiz-Palacios, O. and V. Valcárcel. 2013. From Messinian crisis to Mediterranean climate: A temporal gap of diversification recovered from multiple plant phylogenies. Perspect. Plant Ecol. Evol. Syst. **15**:130-137.
Flemons, P., R. Guralnick, J. Krieger, A. Ranipeta, and D. Neufeld. 2007. A web-based GIS tool for exploring the world's biodiversity: The Global Biodiversity Information Facility Mapping and Analysis Portal Application (GBIF-MAPA). Ecological Informatics **2**:49-60.
Flora of North America Editorial Committee. 1993. Flora of North America North of Mexico, New York and Oxford.
Fonseca, C. R., J. M. Overton, B. Collins, and M. Westoby. 2000. Shifts in trait-combinations along rainfall and phosphorus gradients. Journal of Ecology **88**:964-977.
Fox, M. D. 1995. Present environmental influences on the Australian flora. Pages 205-250 *in* A. E. Orchard, editor. Flora of Australia. ABRS/CSIRO Australia, Melbourne.
Friis, E. M., K. R. Pedersen, and P. R. Crane. 2006. Cretaceous angiosperm flowers: Innovation and evolution in plant reproduction. Palaeogeography, Palaeoclimatology, Palaeoecology **232**:251-293.
Galley, C., B. Bytebier, D. U. Bellstedt, and H. P. Linder. 2007. The Cape element in the Afrotemperate flora: from Cape to Cairo? Proc. R. Soc. Lond. B **274**:535-543.
Galley, C. and H. P. Linder. 2006. Geographical affinities of the Cape flora, South Africa. J. Biogeogr. **33**:236-250.
Gillespie, E. and K. Kron. 2010. Molecular phylogenetic relationships and a revised classification of the subfamily Ericoideae (Ericaceae). Molecular Phylogenetics and Evolution **56**:343-354.
Givnish, T. J. 1997. Adaptive radiations and molecular systematics: issues and approaches. Pages 1-54 *in* T. J. Gisnish and S. K.J., editors. Molecular evolution and adaptive radiation. Cambridge University Press, Cambridge, UK.

Givnish, T. J. 2010. Ecology of plant speciation. Taxon **59**:1326-1366.
Givnish, T. J., M. Ames, J. R. McNeal, M. R. McKain, P. R. Steele, C. W. dePamphilis, S. W. Graham, J. C. Pires, D. W. Stevenson, W. B. Zomlefer, B. G. Briggs, M. R. Duvall, M. J. Moore, J. M. Heaney, D. E. Soltis, P. S. Soltis, K. Thiele, and J. H. Leebens-Mack. 2010. Assembling the tree of the Monocotyledons: Plastome sequence phylogeny and evolution of Poales. Annals of the Missouri Botanical Garden **97**:584-616.
Givnish, T. J., M. H. J. Barfuss, B. V. Ee, R. Riina, K. Schulte, R. Horres, P. A. Gonsiska, R. S. Jabaily, D. M. Crayn, J. A. C. Smith, K. Winter, G. K. Brown, T. M. Evans, B. K. Holst, H. Luther, W. Till, G. Zizka, P. E. Berry, and K. J. Sytsma. 2014. Adaptive radiation, correlated and contingent evolution, and net species diversification in Bromeliaceae. Molecular Phylogenetics and Evolution **71**:55-78.
Givnish, T. J., T. M. Evans, J. C. Pires, and K. J. Sytsma. 1999. Polyphyly and convergent morphological evolution in Commelinales and Commelinidae: Evidence from rbcL sequence data. Molecular Phylogenetics and Evolution **12**:360-385.
Glor, R. E. 2010. Phylogenetic insights on adaptive radiation. Annual Review of Ecology, Evolution, and Systematics **41**:251-270.
Goldberg, E. E., L. T. Lancaster, and R. H. Ree. 2011. Phylogenetic inference of reciprocal effects between geographic range evolution and diversification. Syst. Biol. **60**:451-465.
Goldblatt, P. 1978. An Analysis of the Flora of Southern Africa: Its Characteristics, Relationships, and Orgins. Ann. Missouri Bot. Gard. **65**:369-436.
Goldblatt, P. and J. C. Manning. 2002. Plant diversity of the Cape Region of Southern Africa. Annals of the Missouri Botanical Garden **89**:281-302.
Goodland, R. and R. Pollard. 1973. The Brazilian cerrado vegetation: a fertility gradient. Journal of Ecology **61**:219-224.
Gould, S. J. and N. Eldredge. 1977. Punctuated Equilibria: The Tempo and Mode of Evolution Reconsidered. Paleobiology **3**:115-151.
Gould, S. J. and E. S. Vrba. 1982. Exaptation-a missing term in the science of form. Paleobiology **8**:4-15.
Gradstein, F. M., J. G. Ogg, A. G. Smith, and e. al. 2004. A geologic timescale. Cambridge University Press, Cambridge (39 co-authors).
Greuter, W. 1994. Extinctions in Mediterranean Areas. Phil. Trans. R. Soc. Lond. B **344**:41-46.
Groppo, M., J. R. Pirani, M. L. F. Salatino, S. R. Blanco, and J. A. Kallunki. 2008. Phylogeny of Rutaceae based on twononcoding regions from cpDNA. American Journal of Botany **95**:985-1005.
Guyer, C. and J. B. Slowinski. 1993. Adaptive Radiation and the Topology of Large Phylogenies. Evolution **47**:253-263.
Hamberlandt, D. 1904. Physiologische Pflanzenanatomie. Engelmann, Leipzig, Germany.
Hämmerli, S. unpublished. Taxonomic revision of *Phylica* (Rhamnaceae) Ph.D. Thesis. University of Zürich, Zürich, Switzerland.
Hansen, T. F. 1997. Stabilizing Selection and the Comparative Analysis of Adaptation. Evolution **51**:1341-1351.
Hardy, C. and H. Linder. 2005. Reconstructing ancestral habitats and ecologies: accounting for intraspecific variability and issues of timing in ecological diversification. Systematic Biology **54**:299-316.
Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008. GEIGER: investigating evolutionary radiations. Bioinformatics **24**:129-131.
Heard, S. B. and D. L. Hauser. 1995. Key evolutionary innovations and their ecological mechanisms. Historical Biology **10**:151-173.
Heled, J. and A. J. Drummond. 2011. Calibrated tree priors for relaxed phylogenetics and divergence time estimation. Syst. Biol. **61**:138-149.
Herrera, C. M. 1992. Historical effects and sorting processes as explanations for contemporary ecological patterns: character syndromes in Mediterranean woody plants. Am. Nat. **140**:421-446.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology **25**:1965-1978.
Hill, R. S. 1998. Fossil evidence for the onset of xeromorphy and scleromorphy in Australian Proteaceae. Australian Systematic Botany **11**:391-400.
Hobbs, R. J., D. M. Richardson, and G. W. Davis. 1995. Mediterranean-type ecosystems: Opportunities and constraints for studying the function of biodiversity. Pages l-42 *in* G. W. Davis and D. M. Richardson, editors. Mediterranean-Type Ecosystems: The Function of Biodiversity. Springer-Verlag.
Hodges, S. A. 1997. Floral Nectar Spurs and Diversification. Int. J. Plant Sci. **158**:S81-S88.
Hodges, S. A. and M. L. Arnold. 1995a. Spurring Plant Diversification: Are Floral Nectar Spurs a Key Innovation? Proceedings of the Royal Society of London. Series B: Biological Sciences **262**:343-348.
Hodges, S. A. and M. L. Arnold. 1995b. Spurring Plant Diversification: Are Floral Nectar Spurs a Key Innovation? Proc. R. Soc. Lond. B **262**:343-348.
Hoetzel, S., L. Dupont, E. Schefusz, F. Rommerskirchen, and G. Wefer. 2013. The role of fire in Miocene to Pliocene C4 grassland and ecosystem evolution. Nature Geosci **6**:1027-1030.
Holmes, G. D., T. L. Downing, E. A. James, M. J. Blacket, A. A. Hoffmann, and M. J. Bayly. 2014. Phylogeny of the holly grevilleas (Proteaceae) based on nuclear ribosomal and chloroplast DNA. Australian Systematic Botany **27**:56-77.
Holst, B. K. 1997. Bromeliaceae. Flora of the Venezuelan guayana. Missouri Botanical Gardens., St Louis.
Holt, R. D. 2009. Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. Proc. Natl. Acad. Sci. U.S.A. **106**:19659-19665.
Hopper, S. 2009. OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. Plant and Soil **322**:49-86.
Hopper, S. D. and P. Gioia. 2004. The Southwest Australian Floristic Region: evolution and conservation of a global hot spot of biodiversity. Annual Review of Ecology, Evolution, and Systematics **35**:623-650.
Hopper, S. D., R. J. Smith, M. F. Fay, J. C. Manning, and M. W. Chase. 2009. Molecular phylogenetics of Haemodoraceae in the Greater Cape and Southwest Australian Floristic Regions. Mol. Phylogenet. Evol. **51**:19-30.
Huang, H. and D. L. Rabosky. 2014. Sexual Selection and Diversification: Reexamining the Correlation between Dichromatism and Speciation Rate in Birds. The American Naturalist **184**:E101-E114.
Huber, M. and A. Goldner. 2012. Eocene monsoons. Journal of Asian Earth Sciences **44**:3-23.
Huff, P. M., P. Wilf, and E. J. Azumah. 2003. Digital Future for Paleoclimate Estimation from Fossil Leaves? Preliminary Results. PALAIOS **18**:266-274.
Hughes, C. and R. Eastwood. 2006. Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. Proc. Natl. Acad. Sci. U.S.A. **103**:10334-10339.
Hughes, C. E. and G. W. Atchison. 2015. The ubiquity of alpine plant radiations: from the Andes to the Hengduan Mountains. New. Phytol.:n/a-n/a.
Hughes, N. F. and A. B. McDougall. 1990. Barremian-Aptian angiospermid pollen records from southern England. Review of Palaeobotany and Palynology **65**:145-151.
Hutchinson, G. E. 1957. Concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology **22**:415-427.
InsideWood. 2004 onwards. Published on the Internet. http://insidewood.lib.ncsu.edu/.
Islam, M. B. and M. P. Simmons. 2006. A Thorny Dilemma: Testing Alternative Intrageneric Classifications within Ziziphus (Rhamnaceae). Syst. Bot. **31**:826-842.
Jacobs, B. F., J. D. Kingston, and L. L. Jacobs. 1999. The origin of grass-dominated ecosystems. Annals of the Missouri Botanical Garden **86**:590-643.
Janssens, S. B., E. B. Knox, S. Huysmans, E. F. Smets, and V. S. F. T. Merckx. 2009. Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: Result of a global climate change. Molecular Phylogenetics and Evolution **52**:806-824.

Jansson, R. and M. Dynesius. 2002. The Fate of Clades in a World of Recurrent Climatic Change: Milankovitch Oscillations and Evolution. Annu. Rev. Ecol. Evol. Syst. **33**:741-777.
Jaramillo, C., M. J. Rueda, and G. Mora. 2006. Cenozoic Plant Diversity in the Neotropics. Science **311**:1893-1896.
Jardiné, S. and I. Magloire. 1965. Palynologie et stratigraphic du Cretace des Basins du Senegal et de Cote d' Ivoire. Ler Coll. African Micropali., Dakar, Mem. Bur. Rech. Geol. Min. **32**:187-245.
Jiménez, I. and R. E. Ricklefs. 2014. Diversity anomalies and spatial climate heterogeneity. Global Ecology and Biogeography **23**:988-999.
Johnson, L. A. S. and B. G. Briggs. 1975. On the Proteaceae—the evolution and classification of a southern family*. Botanical Journal of the Linnean Society **70**:83-182.
Johnson, S. D. 1996. Pollination, Adaptation and Speciation Models in the Cape Flora of South Africa. Taxon **45**:59-66.
Jones, C. S., F. T. Bakker, C. D. Schlichting, and A. B. Nicotra. 2009. Leaf shape evolution in the South African genus *Pelargonium* L'Her (Geraniaceae). Evolution **63**:479-497.
Jordan, G. J. 1995. Early-Middle Pleistocene leaves of extinct and extant Proteaceae from western Tasmania, Australia. Botanical Journal of the Linnean Society **118**:19-35.
Jordan, G. J., T. J. Brodribb, C. J. Blackman, and P. H. Weston. 2013. Climate drives vein anatomy in Proteaceae. American Journal of Botany **100**:1483-1493.
Jordan, G. J., R. J. Carpenter, and R. S. Hill. 1998. The macrofossil record of Proteaceae in Tasmania: a review with new species. Australian Systematic Botany **11**:465-501.
Jordan, G. J., R. J. Carpenter, A. Koutoulis, A. Price, and T. J. Brodribb. 2015. Environmental adaptation in stomatal size independent of the effects of genome size. New Phytologist **205**:608-617.
Jordan, G. J., R. A. Dillon, and P. H. Weston. 2005. Solar radiation as a factor in the evolution of scleromorphic leaf anatomy in Proteaceae. American Journal of Botany **92**:789-796.
Jordan, G. J. and R. S. Hill. 1996. The fossil record of the Epacridaceae. Annals of Botany **77**:341-346.
Jordan, G. J., P. H. Weston, R. J. Carpenter, R. A. Dillon, and T. J. Brodribb. 2008. The evolutionary relations of sunken, covered, and encrypted stomata to dry habitats in Proteaceae. Am. J. Bot. **95**:521-530.
Kattge, J. and S. Díaz and S. Lavorel and I. C. Prentice and P. Leadley and G. Bönisch and E. Garnier and M. Westoby and P. B. Reich and I. J. Wright and J. H. C. Cornelissen and C. Violle and S. P. Harrison and P. M. Van Bodegom and M. Reichstein and B. J. Enquist and N. A. Soudzilovskaia and D. D. Ackerly and M. Anand and O. Atkin and M. Bahn and T. R. Baker and D. Baldocchi and R. Bekker and C. C. Blanco and B. Blonder and W. J. Bond and R. Bradstock and D. E. Bunker and F. Casanoves and J. Cavender-Bares and J. Q. Chambers and F. S. Chapin Iii and J. Chave and D. Coomes and W. K. Cornwell and J. M. Craine and B. H. Dobrin and L. Duarte and W. Durka and J. Elser and G. Esser and M. Estiarte and W. F. Fagan and J. Fang and F. FernÁNdez-MÉNdez and A. Fidelis and B. Finegan and O. Flores and H. Ford and D. Frank and G. T. Freschet and N. M. Fyllas and R. V. Gallagher and W. A. Green and A. G. Gutierrez and T. Hickler and S. I. Higgins and J. G. Hodgson and A. Jalili and S. Jansen and C. A. Joly and A. J. Kerkhoff and D. Kirkup and K. Kitajima and M. Kleyer and S. Klotz and J. M. H. Knops and K. Kramer and I. KÜHn and H. Kurokawa and D. Laughlin and T. D. Lee and M. Leishman and F. Lens and T. Lenz and S. L. Lewis and J. Lloyd and J. LlusiÀ and F. Louault and S. Ma and M. D. Mahecha and P. Manning and T. Massad and B. E. Medlyn and J. Messier and A. T. Moles and S. C. MÜLler and K. Nadrowski and S. Naeem and Ü. Niinemets and S. NÖLlert and A. NÜSke and R. Ogaya and J. Oleksyn and V. G. Onipchenko and Y. Onoda and J. OrdoÑEz and G. Overbeck and W. A. Ozinga and S. PatiÑO and S. Paula and J. G. Pausas and J. PeÑUelas and O. L. Phillips and V. Pillar and H. Poorter and L. Poorter and P. Poschlod and A. Prinzing and R. Proulx and A. Rammig and S. Reinsch and B. Reu and L. Sack and B. Salgado-Negret and J. Sardans and S. Shiodera and B. Shipley and A. Siefert and E. Sosinski and J. F. Soussana and E. Swaine and N. Swenson and K. Thompson and P. Thornton and M. Waldram and E. Weiher and M. White and S. White and S. J. Wright and B. Yguel and S. Zaehle and A. E. Zanne and C. Wirth. 2011. TRY – a global database of plant traits. Global Change Biology **17**:2905-2935.

Keeley, J. E., W. J. Bond, R. A. Bradstock, J. G. Pausas, and P. W. Rundel. 2012. Fire in Mediterranean ecosystems ecology, evolution and management. 1st edition. Cambridge Univ. Press, New York.
Kendall, D. G. 1949. Stochastic Processes and Population Growth. J. R. Stat. Soc. Ser. B Stat. Methodol. **11**:230-282.
Kidwell, S. M. and S. M. Holland. 2002. The quality of the fossil record: implications for evolutionary analyses. Annual Review of Ecology and Systematics **33**:561-588.
Kisel, Y., L. McInnes, N. H. Toomey, and C. D. L. Orme. 2011. How diversification rates and diversity limits combine to create large-scale species–area relationships.
Klak, C., G. Reeves, and T. Hedderson. 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. Nature **427**:63-65.
Klingenberg, C. P. 2011. MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources **11**:353-357.
Kottek, M., J. Grieser, C. Beck, B. Rudolf, and F. Rubel. 2006. World Map of the Köppen-Geiger climate classification updated. Meteorologische Zeitschrift **15**:5.
Kozak, K. H. and J. J. Wiens. 2010. Accelerated rates of climatic-niche evolution underlie rapid species diversification. Ecology Letters **13**:1378-1389.
Kreft, H. and W. Jetz. 2007. Global patterns and determinants of vascular plant diversity. Proc. Natl. Acad. Sci. U.S.A. **104**:5925-5930.
Kron, K. A., W. S. Judd, P. F. Stevens, D. M. Crayn, A. A. Anderberg, P. A. Gadek, C. J. Quinn, and J. L. Luteyn. 2002. Phylogenetic classification of Ericaceae: molecular and morphological evidence. Botanical Review **68**:335-423.
Kruskal, J. B. 1964. Nonmetric multidimensional scaling: a numerical method. Psychometrika **29**:115-129.
Kubitzki, K. 1993. Betulaceae, pp. 152-156, Casuarinaceae, pp. 237-241, Fagaceae (incl Nothofagaceae), 301-309, Juglandaceae -357, Myricaceae, 453-457, and Ticodendraceae, pp. 594-595.*in* K. Kubitzki, J. G. Rohwer, and V. Bittrich, editors. The Families and Genera of Vascular Plants. II. Flowering Plants: Dicotyledons, Magnoliid, Hamamelid and Caryophyllid Families. Springer, Berlin.
Kubitzki, K. 1998. The families and genera of vascular plants. Flowering plants Monocotyledons, Alismatanae and Commelinanae (except Gramineae). Springer-Verlag, Berlin.
Kvaček, Z. 2007. Do extant nearest relatives of thermophile European Cenozoic plant elements reliably reflect climatic signal? Palaeogeography, Palaeoclimatology, Palaeoecology **253**:32-40.
Ladiges, P. Y., J. Kellermann, G. Nelson, C. J. Humphries, and F. Udovicic. 2005. Historical biogeography of Australian Rhamnaceae, tribe Pomaderreae. J. Biogeogr. **32**:1909-1919.
Laetsch, W. M. 1974. The C4 syndrome: a structural analysis. Annual Review of Plant Physiology **25**:27–52.
Lambers, H., M. Brundrett, J. Raven, and S. Hopper. 2010. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. Plant and Soil **334**:11-31.
Lambers, H. and H. Poorter. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences.
Lancaster, L. T. and K. M. Kay. 2013. Origin and diversification of the California flora: re-examining classic hypotheses with molecular phylogenies. Evolution **67**:1041-1054.
Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. Mol. Biol. Evol. **29**:1695-1701.
Lauber, K., G. Wagner, and A. Gygax. 2012. Flora Helvetica. Haupt Verlag, Bern.
Lazarides, M. 1980. The tropical grasses of Southeast Asia. Strauss and Cramer GmbH, Germany.
Levyns, M. R. 1964. Migrations and origin of the Cape flora. Trans. Roy. Soc. S. Afr. **37**:85-107.
Lieberman, B. 2012. Adaptive radiations in the context of macroevolutionary theory: a paleontological perspective. Evolutionary Biology **39**:181-191.
Linder, H. P. 2003. The radiation of the Cape flora, southern Africa. Biol. Rev. **78**:597.
Linder, H. P. 2005. Evolution of diversity: the Cape flora. Trends Plant Sci. **10**:536-541.

Linder, H. P. 2008. Plant species radiations: where, when, why? Philosophical Transactions of the Royal Society B: Biological Sciences **363**:3097-3105.
Linder, H. P., O. Bykova, J. Dyke, R. S. Etienne, T. Hickler, I. Kühn, G. Marion, R. Ohlemüller, S. J. Schymanski, and A. Singer. 2012. Biotic modifiers, environmental modulation and species distribution models. J. Biogeogr. **39**:2179–2190.
Linder, H. P. and C. R. Hardy. 2004. Evolution of the species-rich Cape flora. Phil. Trans. R. Soc. Lond. B **359**:1623-1632.
Linder, H. P., D. L. Rabosky, A. Antonelli, R. O. Wüest, and R. Ohlemüller. 2014. Disentangling the influence of climatic and geological changes on species radiations. J. Biogeogr. **41**:1313-1325.
Litsios, G., R. O. Wüest, A. Kostikova, F. Forest, C. Lexer, H. P. Linder, P. B. Pearman, N. E. Zimmermann, and N. Salamin. 2014. Effects of a fire response trait on diversification in replicated radiations. Evolution **68**:453–465.
Losos, J. B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecology Letters **11**:995-1003.
Losos, J. B. 2009. Lizards in an evolutionary tree: ecology and adaptive radiation of anoles. . University California Press, Berkely.
Losos, Jonathan B. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. The American Naturalist **175**:623-639.
Losos, J. B. 2011. Convergence, adaptation, and constraint. Evolution **65**:1827-1840.
Losos, J. B. and D. L. Mahler. 2010. Adaptive radiation: the interaction of ecological opportunity, adaptation, and speciation. Evolution since Darwin: the first **150**:381-420.
MacGinitie, H. D. 1953. Fossil plants of the Florissant beds, Colorado. Carnegie Institution of Washington.
Maddison, W. P. 2006. Confounding asymmetries in evolutionary diversification and character change. Evolution **60**:1743-1746.
Maddison, W. P., P. E. Midford, and S. P. Otto. 2007. Estimating a binary character's effect on speciation and extinction. Systematic Biology **56**:701-710.
Madriñán, S., A. J. Cortés, and J. E. Richardson. 2013. Páramo is the world's fastest evolving and coolest biodiversity hotspot. Front. Genet. **4**.
Magallon, S. and A. Castillo. 2009. Angiosperm diversification through time. Am. J. Bot. **96**:349-365.
Magallón, S., S. Gómez-Acevedo, L. L. Sánchez-Reyes, and T. Hernández-Hernández. 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. New. Phytol.:DOI: 10.1111/nph.13264.
Magallon, S. and M. Sanderson. 2001. Absolute diversification rates in angiosperm clades. Evolution **55**:1762 - 1780.
Maharjan, S. K., L. Poorter, M. Holmgren, F. Bongers, J. J. Wieringa, and W. D. Hawthorne. 2011. Plant functional traits and the distribution of West African rain forest trees along the rainfall gradient. Biotropica **43**:552-561.
Mahler, D. L., L. J. Revell, R. E. Glor, and J. B. Losos. 2010. Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean Anoles. Evolution **64**:2731-2745.
Makinson, R. 2000. Grevillea. Pages 1-460 *in* A. Wilson, editor. Flora of Australia. CSIRO Publishing, Melbourne.
Manchester, S. R. 2001. Update on the megafossil flora of Florissant, Colorado, USA. Pages 137–161 *in* E. Evanoff, K. M. Gregory-Wodzicki, and K. R. Johnson, editors. Fossil flora and stratigraphy of the Florissant Formation, Colorado: Proc. denver Mus. Nature & Science.
Manning, J. and P. Goldblatt. 2013. Plants of the Greater Cape Floristic Region 1: The Core Cape flora. Strelitzia 29, Pretoria.
Marazzi, B., C. Ané, M. F. Simon, A. Delgado-Salinas, M. Luckow, and M. J. Sanderson. 2012. Locating evolutionary precursors on a phylogenetic tree. Evolution **66**:3918-3930.
Markgraf, V., M. McGlone, and G. Hope. 1995. Neogene paleoenvironmental and paleoclimatic change in southern temperate ecosystems — a southern perspective. Trends Ecol. Evol. **10**:143-147.

Martin, A. R. H. 1995. Palaeogene proteaceous pollen and phylogeny. Alcheringa: An Australasian Journal of Palaeontology **19**:27-40.
Martin, A. R. H. and W. K. Harris. 1974. Reappraisal of some palynomorphs of supposed Proteaceous affinity. Grana **14**:108-113.
Martin, H. A. 2006. Cenozoic climatic change and the development of the arid vegetation in Australia. Journal of Arid Environments **66**:533-563.
Martínez-Cabrera, H. I., C. D. Schlichting, J. A. Silander, and C. S. Jones. 2012. Low levels of climate niche conservatism may explain clade diversity patterns in the South African genus Pelargonium (Geraniaceae). American Journal of Botany **99**:954-960.
Mast, A. R., E. F. Milton, E. H. Jones, R. M. Barker, W. R. Barker, and P. H. Weston. 2012. Time-calibrated phylogeny of the woody Australian genus Hakea (Proteaceae) supports multiple origins of insect-pollination among bird-pollinated ancestors. American Journal of Botany **99**:472-487.
McDonald, P. G., C. R. Fonseca, J. M. Overton, and M. Westoby. 2003. Leaf-Size Divergence along Rainfall and Soil-Nutrient Gradients: Is the Method of Size Reduction Common among Clades? Functional Ecology **17**:50-57.
McGuire, A. F. and K. A. Kron. 2005. Phylogenetic relationships of European and African ericas. International Journal of Plant Sciences **166**:311-318.
McLellan, T. and J. A. Endler. 1998. The relative success of some methods for measuring and describing the shape of complex objects. Systematic Biology **47**:264-281.
McLoughlin, S. and R. S. Hill. 1996. The succession of Western Australian phanerozoic terrestrial floras. . Pages 61-80 *in* H. S.D., C. J.A., H. M.S., and G. A.S., editors. Gondwanan heritage: past, present and future of the Western Australian biota. Australia Surrey Beatty and Sons Pty Ltd, Chipping Norton, NSW, Australia.
McPeek, M. A. and J. M. Brown. 2007. Clade age and not diversification rate explains species richness among animal taxa. Am. Nat. **169**:E97-E106.
Meadows, M. and J. Sugden. 1993. The late quaternary palaeoecology of a floristic kingdom: the southwestern Cape South Africa. Palaeogeogr. Palaeoclimatol. Palaeoecol. **101**:271-281.
Medan, D. and C. Schirarend. 2004. Rhamnaceae. Pages 320-338 *in* K. Kubitzki, editor. The Families and Genera of Vascular Plants. Springer-Verlag, Berlin.
Meers, T. L., T. L. Bell, N. J. Enright, and S. Kasel. 2008. Role of plant functional traits in determining vegetation composition of abandoned grazing land in north-eastern Victoria, Australia. Journal of Vegetation Science **19**:515-524.
Midgley, G. 2012. Biodiversity and ecosystem function. Science **335**:174-175.
Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pages 1-8 *in* Gateway Computing Environments Workshop (GCE), 2010.
Milne, L. A. 1994. Relationship between *Propylipollis annularis* (Tertiary dispersed pollen) and extant Xylomelum (Proteaceae). Pages 193-213 *in* M. H. Kurmann and J. A. Doyle, editors. Ultrastructure of Fossil Spores and Pollen. Royal Botanic Gardens, Kew.
Milne, L. A. 1998. Tertiary palynology: *Beaupreaidites* and new Conospermeae (Proteoideae) affiliates. Australian Systematic Botany **11**:553-603.
Mitchell, N., T. E. Moore, H. K. Mollmann, J. E. Carlson, K. Mocko, H. Martinez-Cabrera, C. Adams, J. A. S. Jr, C. S. Jones, C. D. Schlichting, and K. E. Holsinger. 2015. Functional traits in parallel evolutionary radiations and trait-environment associations in the Cape Floristic Region of South Africa. The American Naturalist **185**:525-537.
Mittelbach, G. G., D. W. Schemske, H. V. Cornell, A. P. Allen, J. M. Brown, M. B. Bush, S. P. Harrison, A. H. Hurlbert, N. Knowlton, H. A. Lessios, C. M. McCain, A. R. McCune, L. A. McDade, M. A. McPeek, T. J. Near, T. D. Price, R. E. Ricklefs, K. Roy, D. F. Sax, D. Schluter, J. M. Sobel, and M. Turelli. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecology Letters **10**:315-331.
Mitter, C., B. Farrell, and B. Wiegmann. 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? The American Naturalist **132**:107-128.

Mittermeier, R. A., P. R. Gil, M. Hoffmann, J. D. Pilgrim, T. M. Brooks, C. G. Mittermeier, J. F. Lamoreux, and G. A. B. da Fonseca. 2004. Hotspots revisited: earth's biologically richest and most endangered terrestrial ecoregions. Sierra Madre (CA): University of Virginia, .
Moen, D. and H. Morlon. 2014. From dinosaurs to modern bird diversity: extending the time scale of adaptive radiation. PLoS Biology **12**:e1001854.
Moldenke, H. N. 1955. Flore de Madagascar Eriocaulaceae. Typographie Firmin-Didot, Paris.
Mooney, H. A. and E. L. Dunn. 1970. Convergent evolution of Mediterranean-climate evergreen sclerophyll shrubs. Evolution **24**:292-303.
Moore, B. R. and M. J. Donoghue. 2007. Correlates of diversification in the plant clade Dipsacales: geographic movement and evolutionary innovations. Am. Nat. **170**:S28-S55.
Moran, P. A. P. 1951. Estimation Methods for Evolutive Processes. J. R. Stat. Soc. Ser. B Stat. Methodol. **13**:141-146.
Moreau, C. S., C. D. Bell, R. Vila, S. B. Archibald, and N. E. Pierce. 2006. Phylogeny of the ants: diversification in the age of angiosperms. Science **312**:101-104.
Morlon, H. 2014. Phylogenetic approaches for studying diversification. Ecology Letters **17**:508-525.
Mosbrugger, V., T. Utescher, and D. L. Dilcher. 2005. Cenozoic continental climatic evolution of Central Europe. Proc. Natl. Acad. Sci. U.S.A. **102**:14964-14969.
Mucina, L. and C. J. Geldenhuys. 2006. Afrotemperate, subtropical and Azonal forests.*in* L. Mucina and M. C. Rutherford, editors. The vegetation of South Africa, Lesotho and Swaziland. Pretoria : South African National Biodiversity Institute.
Mueller. 1873. New vegetable fossils of Victoria. Reports of mining Surveyors and Registrars, Victoria **Quarter ending 30 September, 1873**:41-42.
Mummenhoff, K., I. A. Al-Shehbaz, F. T. Bakker, H. P. Linder, and A. Mühlhausen. 2005. Phylogeny, morphological evolution, and speciation of endemic Brassicaceae genera in the Cape flora of Southern Africa. Ann. Missouri Bot. Gard. **92**:400-424.
Nagalingum, N. S., C. R. Marshall, T. B. Quental, H. S. Rai, D. P. Little, and S. Mathews. 2011. Recent synchronous radiation of a living fossil. Science **334**:796-799.
NatureServe Explorer. 2002. An online encyclopedia of life [web application]. 2001. Version 1.6. NatureServe. [accessed November 2013], Arlington, Virginia, USA.
Near, T. J., A. Dornburg, K. L. Kuhn, J. T. Eastman, J. N. Pennington, T. Patarnello, L. Zane, D. A. Fernández, and C. D. Jones. 2012. Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. Proc. Natl. Acad. Sci. U.S.A. **109**:3434-3439.
Nee, S., E. C. Holmes, R. M. May, and P. H. Harvey. 1994. Extinction Rates can be Estimated from Molecular Phylogenies. Philosophical Transactions: Biological Sciences **344**:77-82.
Nobel, P. S. 1983. Biophysical plant physiology and ecology. W.H. Freeman, San Francisco.
Nylander, J. A. A., J. C. Wilgenbusch, D. L. Warren, and D. L. Swofford. 2008. AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics **24**:581-583.
O'Leary, M. A., J. I. Bloch, J. J. Flynn, T. J. Gaudin, A. Giallombardo, N. P. Giannini, S. L. Goldberg, B. P. Kraatz, Z.-X. Luo, J. Meng, X. Ni, M. J. Novacek, F. A. Perini, Z. S. Randall, G. W. Rougier, E. J. Sargis, M. T. Silcox, N. B. Simmons, M. Spaulding, P. M. Velazco, M. Weksler, J. R. Wible, and A. L. Cirranello. 2013. The placental mammal ancestor and the post–K-Pg radiation of placentals. Science **339**:662-667.
Olde, P. and N. Marriott. 1994. The Grevillea book: volume 1. Kangaroo Press, Sydney.
Onstein, R. E., R. J. Carter, Y. Xing, and H. P. Linder. 2014. Diversification rate shifts in the Cape Floristic Region: The right traits in the right place at the right time. Perspectives in Plant Ecology, Evolution and Systematics **16**:331-340.
Onstein, R. E., R. J. Carter, Y. Xing, J. E. Richardson, and H. P. Linder. 2015. Do Mediterranean-type ecosystems have a common history? – Insights from the Buckthorn family (Rhamnaceae). Evolution **69**:756-771.
Ordoñez, J. C., P. M. Van Bodegom, J.-P. M. Witte, I. J. Wright, P. B. Reich, and R. Aerts. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecol. Biogeogr. **18**:137-149.

Orme, D., R. Freckleton, G. Thomas, T. Petzoldt, S. Fritz, N. Isaac, and W. Pearse. 2013. Caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5.2. http://CRAN.R-project.org/package=caper.

Pagel, M. 1994. Detecting Correlated Evolution on Phylogenies: A General Method for the Comparative Analysis of Discrete Characters. Proc. R. Soc. B **255**:37-45.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature **401**:877-884.

Pagel, M. and A. Meade. 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump markov chain monte carlo. The American Naturalist **167**:808-825.

Paradis, E. 2005. Statistical analysis of diversification with species traits. Evolution **59**:1-12.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics **20**:289-290.

Parkhurst, D. F. and O. L. Loucks. 1972. Optimal leaf size in relation to environment. Journal of Ecology **60**:505-537.

Partridge, T. C. and R. R. Maud. 1987. Geomorphic evolution of southern Africa since the Mesozoic. . S. Afr. J. Geol. **90**:179-208.

Pearman, P. B., A. Guisan, O. Broennimann, and C. F. Randin. 2008. Niche dynamics in space and time. Trends Ecol. Evol. **23**:149-158.

Pennington, R. T., J. E. Richardson, and M. Lavin. 2006. Insights into the historical construction of species-rich biomes from dated plant phylogenies, neutral ecological theory and phylogenetic community structure. New Phytologist **172**:605-616.

Pennington, T. D., C. Reynel, and A. Daza. 2004. Illustrated guide to the trees of Peru. D. Hunt, England.

Potter, P. E. and P. Szatmari. 2009. Global Miocene tectonics and the modern world. Earth Sci. Rev. **96**:279-295.

Preacher, K. J. 2001. Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence [Computer software]. Available from http://quantpsy.org.

Prinzing, A. 2001. The niche of higher plants: evidence for phylogenetic conservatism. Proc. R. Soc. Lond. B **268**:2383-2389.

Pybus, O. G. and P. H. Harvey. 2000. Testing macro–evolutionary models using incomplete molecular phylogenies. Proceedings of the Royal Society B: Biological Sciences **267**:2267-2272.

Pyšek, P., T. Kučera, and V. Jarošík. 2002. Plant species richness of nature reserves: the interplay of area, climate and habitat in a central European landscape. Global Ecology and Biogeography **11**:279-289.

R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing. http://www.R-project.org. Vienna, Austria.

Rabosky, D. L. 2010. Extinction rates should not be estimated from molecular phylogenies. Evolution **64**:1816-1824.

Rabosky, D. L. 2014. Automatic detection of key Innovations, rate shifts, and diversity-dependence on phylogenetic trees. PLoS ONE **9**:e89543.

Rabosky, D. L., S. C. Donnellan, M. Grundler, and I. J. Lovette. 2014a. Analysis and Visualization of Complex Macroevolutionary Dynamics: An Example from Australian Scincid Lizards. Systematic Biology **63**:610-627.

Rabosky, D. L., M. Grundler, C. Anderson, P. Title, J. J. Shi, J. W. Brown, H. Huang, and J. G. Larson. 2014b. BAMMtools: an R package for the analysis of evolutionary dynamics on phylogenetic trees. Methods in Ecology and Evolution **5**:701-707.

Rabosky, D. L., F. Santini, J. Eastman, S. A. Smith, B. Sidlauskas, J. Chang, and M. E. Alfaro. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nat Commun **4**.

Rambal, S. 2001. 14 - Hierarchy and Productivity of Mediterranean-Type Ecosystems. Pages 315-344 *in* J. Roy, B. Saugier, and H. A. Mooney, editors. Terrestrial Global Productivity. Academic Press, San Diego.

Rambaut, A. and A. Drummond. 2007. Tracer v1.5. http://beast.bio.ed.ac.uk/Tracer.

Read, D. J. 1996. The structure and function of the ericoid mycorrhizal root. Annals of Botany **77**:365-374.
Rebelo, A., C. Boucher, N. Helme, L. Mucina, and M. Rutherford. 2006. Fynbos biome.*in* L. Mucina and M. C. Rutherford, editors. The vegetation of South Africa, Lesotho and Swaziland. Pretoria : South African National Biodiversity Institute.
Rebelo, A. G. 2006. Protea atlas project. http://protea.worldonline.co.za/default.htm. Accessed 6 August 2013.
Reich, P. B., I. J. Wright, J. Cavender-Bares, J. M. Craine, J. Oleksyn, M. Westoby, and M. B. Walters. 2003. The evolution of plant functional variation: traits, spectra, and strategies. Int. J. Plant Sci. **164**:S143-S164.
Reid, C. and E. M. Reid. 1915. The Pliocene Floras of the Dutch-Prussian Border. Mededeel. van de Rijisopsp. van Delfstoffen, The Hague.
Renvoize, S. A. 1984. The grasses of Bahia. Royal Botanic Gardens Kew, UK.
Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. **3**:217-223.
Reyes, E., H. Morlon, and H. Sauquet. 2015. Presence in Mediterranean hotspots and floral symmetry affect speciation and extinction rates in Proteaceae. New. Phytol.:doi: 10.1111/nph.13244.
Richards, M. B., R. M. Cowling, and W. D. Stock. 1997. Soil nutrient dynamics and community boundaries in the Fynbos vegetation of South Africa. Plant Ecology **130**:143-153.
Richardson, J. E., L. W. Chatrou, J. B. Mols, R. H. J. Erkens, and M. D. Pirie. 2004. Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae. Phil. Trans. R. Soc. Lond. B **359**:1495-1508.
Richardson, J. E., M. F. Fay, Q. C. B. Cronk, D. Bowman, and M. W. Chase. 2000a. A phylogenetic analysis of Rhamnaceae using rbcL and trnL-F plastid DNA sequences. Am. J. Bot. **87**:1309-1324.
Richardson, J. E., M. F. Fay, Q. C. B. Cronk, and M. W. Chase. 2000b. A Revision of the Tribal Classification of Rhamnaceae. Kew Bull. **55**:311-340.
Richardson, J. E., R. T. Pennington, T. D. Pennington, and P. M. Hollingsworth. 2001a. Rapid Diversification of a Species-Rich Genus of Neotropical Rain Forest Trees. Science **293**:2242-2245.
Richardson, J. E., F. M. Weitz, M. F. Fay, Q. C. B. Cronk, H. P. Linder, G. Reeves, and M. W. Chase. 2001b. Phylogenetic analysis of *Phylica* L. (Rhamnaceae) with an emphasis on island species: evidence from plastid trnL-F and nuclear Internal Transcribed Spacer (ribosomal) DNA sequences. Taxon **50**:405-427.
Ricklefs, R. E. 2004. Cladogenesis and morphological diversification in passerine birds. Nature **430**:338-341.
Ricklefs, R. E. 2006. Evolutionary diversification and the origin of the diversity-environment relationship. Ecology **87**:S3-S13.
Roquet, C., F. C. Boucher, W. Thuiller, and S. Lavergne. 2013. Replicated radiations of the alpine genus Androsace (Primulaceae) driven by range expansion and convergent key innovations. Journal of Biogeography **40**:1874-1886.
Rosenblum, E., B. J. Sarver, J. Brown, S. Des Roches, K. Hardwick, T. Hether, J. Eastman, M. Pennell, and L. Harmon. 2012. Goldilocks meets Santa Rosalia: an ephemeral speciation model explains patterns of diversification across time scales. BMC Evol. Biol. **39**:255-261.
Rosenzweig, M. L. 1995. Species diversity in space and time. Cambridge University Press, Cambridge, UK.
Royer, D. L., P. Wilf, D. A. Janesko, E. A. Kowalski, and D. L. Dilcher. 2005. Correlations of climate and plant ecology to leaf size and shape: potential proxies for the fossil record. Am. J. Bot. **92**:1141-1151.
Rudall, P. J. and R. M. Bateman. 2002. Roles of synorganisation, zygomorphy and heterotopy in floral evolution: the gynostemium and labellum of orchids and other lilioid monocots. Biological Reviews **77**:403-441.
Rutschmann, F., T. Eriksson, K. A. Salim, and E. Conti. 2007. Assessing calibration uncertainty in molecular dating: the assignment of fossils to alternative calibration points. Syst. Biol. **56**:591-608.

Rutschmann, F. K. 2006. The uses of molecular dating analyses in evolutionary studies, with examples from the Angiosperms. Ph.D. Thesis. University of Zürich, Zürich, Switzerland.
Rydberg, P. A. 1954. Flora of the Rocky mountains and adjacent plains. 2nd Edition. Hafner Publishing Co, New York.
Sage, R. F. 2004. The evolution of C4 photosynthesis. New. Phytol. **161**:341-370.
Salmon, J. T. 1968. A field guide to the Alpine plants of New Zealand. Reed, Wellington.
Salvo, G., S. Y. W. Ho, G. Rosenbaum, R. Ree, and E. Conti. 2010. Tracing the temporal and spatial origins of island endemics in the Mediterranean Region: a case study from the Citrus family (*Ruta* L., Rutaceae). Syst. Biol. **59**:705-722.
Sanderson, M. J. and M. J. Donoghue. 1994. Shifts in diversification rate with the origin of angiosperms. Science **264**:1590-1593.
Sauquet, H., S. Y. W. Ho, M. A. Gandolfo, G. J. Jordan, P. Wilf, D. J. Cantrill, M. J. Bayly, L. Bromham, G. K. Brown, R. J. Carpenter, D. M. Lee, D. J. Murphy, J. M. K. Sniderman, and F. Udovicic. 2012. Testing the impact of calibration on molecular divergence times using a fossil-rich group: the dase of *Nothofagus* (Fagales). Syst. Biol. **61**:289-313.
Sauquet, H., P. H. Weston, C. L. Anderson, N. P. Barker, D. J. Cantrill, A. R. Mast, and V. Savolainen. 2009. Contrasted patterns of hyperdiversification in Mediterranean hotspots. Proc. Natl. Acad. Sci. U.S.A. **106**:221-225.
Scharf, T. E., A. T. Codilean, M. de Wit, J. D. Jansen, and P. W. Kubik. 2013. Strong rocks sustain ancient postorogenic topography in southern Africa. Geology.
Schimper, A. F. W. 1903. Plant-geography upon a physiological basis. Claredon Press, Oxford.
Schluter, D. 2000. The ecology of adaptive radiation. Oxford University press, New York.
Schmerler, S. B., W. L. Clement, J. M. Beaulieu, D. S. Chatelet, L. Sack, M. J. Donoghue, and E. J. Edwards. 2012. Evolution of leaf form correlates with tropical–temperate transitions in *Viburnum* (Adoxaceae). Proc. R. Soc. Lond. B **279**:3905-3913.
Schneider, H., E. Schuettpelz, K. M. Pryer, R. Cranfill, S. Magallon, and R. Lupia. 2004. Ferns diversified in the shadow of angiosperms. Nature **428**:553-557.
Schnitzler, J., T. G. Barraclough, J. S. Boatwright, P. Goldblatt, J. C. Manning, M. P. Powell, T. Rebelo, and V. Savolainen. 2011. Causes of plant diversification in the Cape biodiversity hotspot of South Africa. Syst. Biol. **60**:343-357.
Schnitzler, J., C. H. Graham, C. F. Dormann, K. Schiffers, and H. Peter Linder. 2012. Climatic niche evolution and species diversification in the Cape flora, South Africa. Journal of Biogeography **39**:2201-2211.
Schönenberger, J. and E. Conti. 2003. Molecular phylogeny and floral evolution of Penaeaceae, Oliniaceae, Rhynchocalycaceae, and Alzateaceae (Myrtales). Am. J. Bot. **90**:293-309.
Schuepp, P. H. 1993. Tansley Review No. 59 Leaf boundary layers. New Phytologist **125**:477-507.
Schwery, O., R. E. Onstein, Y. Bouchenak-Khelladi, Y. Xing, R. J. Carter, and H. P. Linder. 2014. As old as the mountains: the radiations of the Ericaceae. New Phytologist. DOI: 10.1111/nph.13234.
Schwilk, D. W. and D. D. Ackerly. 2001. Flammability and serotiny as strategies: correlated evolution in pines. Oikos **94**:326-336.
Sebola, R. and K. Balkwill. 2009. Numerical phenetic analysis of *Olinia rochetiana sensu lato* (Oliniaceae). Kew Bulletin **64**:95-121.
Segarra-Moragues, J. G. and F. Ojeda. 2010. Postfire response and genetic diversity in *Erica coccinea*: connecting population dynamics and diversification in a biodiversity hotspot. Evolution **64**:3511-3524.
Sepkoski, J. J., Jr. 1979. A Kinetic Model of Phanerozoic Taxonomic Diversity II. Early Phanerozoic Families and Multiple Equilibria. Paleobiology **5**:222-251.
Silvestro, D., G. Zizka, and K. Schulte. 2014. Disentangling the effects of key innovations on the diversification of Bromelioideae (Bromeliaceae). Evolution **68**:163-175.
Simpson, G. G. 1953. The major features of evolution. 1st edition. Columbia Univ. Press, New York.
Small, J. K. 1903. Flora of the Southeastern United States. Small, New York.
Smith, L. B. and W. Till. 1998. Bromeliaceae.*in* K. Kubitzki, editor. The families and genera of vascular plants. Monocotyledons. . Springer-Verlag, Berlin.

Smith, S. and M. Donoghue. 2008a. Rates of molecular evolution are linked to life history in flowering plants. Science **322**:86 - 89.
Smith, S. A. and M. J. Donoghue. 2008b. Rates of Molecular Evolution Are Linked to Life History in Flowering Plants. Science **322**:86-89.
Sniderman, J. M. K., G. J. Jordan, and R. M. Cowling. 2013. Fossil evidence for a hyperdiverse sclerophyll flora under a non–Mediterranean-type climate. Proc. Natl. Acad. Sci. U.S.A. **110**:3423–3428.
Soltis, D. E., V. A. Albert, J. Leebens-Mack, C. D. Bell, A. H. Paterson, C. Zheng, D. Sankoff, C. W. dePamphilis, P. K. Wall, and P. S. Soltis. 2009. Polyploidy and angiosperm diversification. American Journal of Botany **96**:336-348.
Specht, R. 1969. A comparison of the sclerophyllous vegetation characteristic of Mediterranean type climates in France, California, and Southern Australia. I. Structure, morphology, and succession. Australian Journal of Botany **17**:277-292.
Specht, R. 1979. Ecosystems of the world. Vol. 9A. Heathlands and related shrublands: descriptive studies. Elsevier, Amsterdam.
Specht, R. L. and E. J. Moll. 1983. Mediterranean-Type Heathlands and Sclerophyllous Shrublands of the World: An Overview. Pages 41-65 *in* F. J. Kruger, D. T. Mitchell, and J. U. M. Jarvis, editors. Mediterranean-Type Ecosystems: The Role of Nutrients. Springer Berlin Heidelberg.
Spriggs, E. L., P.-A. Christin, and E. J. Edwards. 2014. C4 photosynthesis promoted species diversification during the Miocene grassland expansion. PLoS ONE **9**:e97722.
Stadler, T. 2011. Mammalian phylogeny reveals recent diversification rate shifts. Proceedings of the National Academy of Sciences **108**:6187-6192.
Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics **22**:2688-2690.
Stanley, S. M. 1979. Macroevolution. Pattern and Process. . Freeman, San Francisco.
Stebbins, G. L. 1974. Flowering plants. Evolution above the species level. Belknap Press of Harvard University Press, Cambridge, Massachusetts.
Stenseth, N. C. 1984. The Tropics: Cradle or Museum? Oikos **43**:417-420.
Stover, L. E. and A. D. Partridge. 1973. Tertiary and Late Cretaceous spores and pollen from the Gippsland Basin, southeastern Australia. Proceedings of the Royal Society of Victoria **85**:237-286.
Suc, J.-P. and S.-M. Popescu. 2005. Pollen records and climatic cycles in the North Mediterranean region since 2.7 Ma. Pages 147-158 *in* M. J. Head and P. L. Gibbard, editors. Early-Middle Pleistocene transitions: The land-ocean evidence. Geological Society, London, Special Publications.
Suc, J. P. 1984. Origin and evolution of the Mediterranean vegetation and climate in Europe. Nature **307**:429-432.
Thomas, D. C., M. Hughes, T. Phutthai, W. H. Ardi, S. Rajbhandary, R. Rubite, A. D. Twyford, and J. E. Richardson. 2012. West to east dispersal and subsequent rapid diversification of the mega-diverse genus *Begonia* (Begoniaceae) in the Malesian archipelago. Journal of Biogeography **39**:98-113.
Thomas, J. A., J. J. Welch, R. Lanfear, and L. Bromham. 2010. A generation time effect on the rate of molecular evolution in invertebrates. Mol. Biol. Evol. **27**:1173-1180.
Thrower, N. W. and D. Bradbury. 1973. The Physiography of the Mediterranean Lands with Special Emphasis on California and Chile. Pages 37-52 *in* F. Castri and H. A. Mooney, editors. Mediterranean Type Ecosystems: Origin and Structure. Springer Berlin Heidelberg.
Thuiller, W., G. F. Midgley, M. Rougeti, and R. M. Cowling. 2006. Predicting patterns of plant species richness in megadiverse South Africa. Ecography **29**:733-744.
Thuiller, W., S. Lavorel, G. Midgley, S. Lavergne, and T. Rebelo. 2004. Relating plant traits and species distributions along bioclimatic gradients for 88 *Leucadendron* taxa. Ecology **85**:1688-1699.
Tinker, J., M. de Wit, and R. Brown. 2008. Mesozoic exhumation of the southern Cape, South Africa, quantified using apatite fission track thermochronology. Tectonophysics **455**:77-93.

Trinder-Smith, T. H., H. P. Linder, T. Niet, G. A. Verboom, and T. L. Nowell. 2007. Plastid DNA sequences reveal generic paraphyly within Diosmeae (Rutoideae, Rutaceae). Syst. Bot. **32**:847-855.
Tutin, T. G. 1980. Flora europaea. Cambridge University Press.
Tyson, P. D. and T. C. Partridge. 2000. Evolution of Cenozoic climates. Pages 371-387 *in* T. C. P. and and R. R. Maud, editors. The Cenozoic of Southern Africa. Oxford University Press, Oxford.
Valente, L. M., G. Reeves, J. Schnitzler, I. P. Mason, M. F. Fay, T. G. Rebelo, M. W. Chase, and T. G. Barraclough. 2010a. Diversification of the African genus *Protea* (Proteaceae) in the Cape biodiversity hotspot and beyond: equal rates in different biomes. Evolution **64**:745-760.
Valente, L. M., V. Savolainen, J. C. Manning, P. Goldblatt, and P. Vargas. 2011. Explaining disparities in species richness between Mediterranean floristic regions: a case study in Gladiolus (Iridaceae). Global Ecology and Biogeography **20**:881-892.
Valente, L. M., V. Savolainen, and P. Vargas. 2010b. Unparalleled rates of species diversification in Europe. Proc. R. Soc. B **277**:1489–1496.
Valente, L. M. and P. Vargas. 2013. Contrasting evolutionary hypotheses between two mediterranean-climate floristic hotspots: the Cape of southern Africa and the Mediterranean Basin. J. Biogeogr. **40**:2032-2046.
van der Niet, T. and S. D. Johnson. 2012. Phylogenetic evidence for pollinator-driven diversification of angiosperms. Trends in Ecology & Evolution **27**:353-361.
Van Valen, L. M. 1973. A new evolutionary law. Evolution Theory **1**:1-30.
van Wilgen, B. W., K. B. Higgins, and D. U. Bellstedt. 1990. The role of vegetation structure and fuel chemistry in excluding fire from forest patches in the fire-prone fynbos shrublands of South Africa. J. Ecol. **78**:210-222.
Verboom, G. A., J. K. Archibald, F. T. Bakker, D. U. Bellstedt, F. Conrad, L. L. Dreyer, F. Forest, C. Galley, P. Goldblatt, J. F. Henning, K. Mummenhoff, H. P. Linder, A. M. Muasya, K. C. Oberlander, V. Savolainen, D. A. Snijman, T. v. d. Niet, and T. L. Nowell. 2009. Origin and diversification of the Greater Cape flora: Ancient species repository, hot-bed of recent radiation, or both? Mol. Phylogenet. Evol. **51**:44-53.
Verboom, G. A., H. P. Linder, and W. D. Stock. 2003. Phylogenetics of the grass genus *Ehrharta*: evidence for radiation in the summer-arid zone of the South African Cape. Evolution **57**:1008-1021.
Verdú, M. 2002. Age at maturity and diversification in woody Angiosperms. Evolution **56**:1352-1361.
Verdú, M., P. Dávila, P. García-Fayos, N. Flores-Hernández, and A. Valiente-Banuet. 2003. 'Convergent' traits of mediterranean woody plants belong to pre-mediterranean lineages. Biological Journal of the Linnean Society **78**:415-427.
Verdú, M. and J. G. Pausas. 2007. Fire drives phylogenetic clustering in Mediterranean Basin woody plant communities. J. Ecol. **95**:1316-1323.
Verdú, M. and J. G. Pausas. 2013. Syndrome-driven diversification in a Mediterranean ecosystem. Evolution **67**:1756-1766.
Verdú, M., J. G. Pausas, J. G. Segarra-Moragues, and F. Ojeda. 2007. Burning phylogenies: fire, molecular evolutionary rates, and diversification. Evolution **61**:2195-2204.
Violle, C., M.-L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, and E. Garnier. 2007. Let the concept of trait be functional! Oikos **116**:882-892.
Vrba, E. S. 1985. Ecology and environment: alternative causes of the temporal distribution of evolutionary events. South African Journal of Science **81**:229-236.
Vrba, E. S. 1993. Turnover-pulses, the red queen, and related topics. American Journal of Science **293-A**:418-452.
Wagner, C. E., L. J. Harmon, and O. Seehausen. 2012. Ecological opportunity and sexual selection together predict adaptive radiation. Nature **487**:366-369.
Wagner, W. L., D. R. Herbst, and S. H. Sohmer. 1990. Manual of the flowering plants of Hawaii. . Bishop Museum Press, University of Hawaii.
Wang, H., M. J. Moore, P. S. Soltis, C. D. Bell, S. F. Brockington, R. Alexandre, C. C. Davis, M. Latvis, S. R. Manchester, and D. E. Soltis. 2009. Rosid radiation and the rapid rise of angiosperm-dominated forests. Proc. Natl. Acad. Sci. U.S.A. **106**:3853–3858.

Ward, J. V. and J. A. Doyle. 1994. Ultrastructure of fossil spores and pollen. Pages 161-172 *in* M. H. Kurmann and J. A. Doyle, editors. Royal Botanic Gardens, Kew, UK.
Watson, L. and M. J. Dallwitz. 1992 onwards. The families of flowering plants: descriptions, illustrations, identification, and information retrieval. Version: 4th March 2011. http://delta-intkey.com.
Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annu. Rev. Ecol. Syst. **33**:475-505.
Wells, P. V. 1969. The Relation Between Mode of Reproduction and Extent of Speciation in Woody Genera of the California Chaparral. Evolution **23**:264-267.
Werner, G. D. A., W. K. Cornwell, J. I. Sprent, J. Kattge, and E. T. Kiers. 2014. A single evolutionary innovation drives the deep evolution of symbiotic N2-fixation in angiosperms. Nat Commun **5**.
Weston, P. H. 2007. Proteaceae. Pages 364-404 *in* K. Kubitski, editor. The families and genera of vascular plants. Springer, Berlin, Germany.
Whittall, J. B. and S. A. Hodges. 2007. Pollinator shifts drive increasingly long nectar spurs in columbine flowers. Nature **447**:706-709.
Wiens, J. J. and M. J. Donoghue. 2004. Historical biogeography, ecology and species richness. Trends Ecol. Evol. **19**:639-644.
Wiens, J. J. and M. C. Morrill. 2011. Missing Data in Phylogenetic Analysis: Reconciling Results from Simulations and Empirical Data. Syst. Biol. **60**:719-731.
Wikström, N., V. Savolainen, and M. W. Chase. 2001. Evolution of the angiosperms: calibrating the family tree. Proc. R. Soc. Lond. B **268**:2211-2220.
Wing, S. L. and L. D. Boucher. 1998. Ecological aspects of the Cretaceous flowering plant radiation. Annual Review of Earth and Planetary Sciences **26**:379-421.
Wright, I., P. Reich, M. Westoby, D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. Groom, J. Gulias, K. Hikosaka, B. Lamont, T. Lee, W. Lee, C. Lusk, J. Midgley, M.-L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. Pyankov, C. Roumet, S. Thomas, M. Tjoelker, E. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature **428**:821-827.
Wright, I. J. and K. Cannon. 2001. Relationships between leaf lifespan and structural defences in a low-nutrient, sclerophyll flora. Functional Ecology **15**:351-359.
Wright, I. J., M. Westoby, and P. B. Reich. 2002. Convergence towards higher leaf mass per area in dry and nutrient-poor habitats has different consequences for leaf life span. Journal of Ecology **90**:534-543.
Wu, Z. and P. Raven. 1999. Flora of China. Vol. 4 (Cycadaceae through Fagaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.
Wyrwoll, K.-H., L. T. Turner, and P. Findlater. 2014. 1A. On the origins, geomorphology and soils of the sandplains of South-Western Australia. Pages 3-22 *in* H. Lambers, editor. Plant life on the sandplains in Southwest Austrlia a global biodiversity hotspot. UWA Publishing, Crawley, Western Australia.
Xing, Y., R. E. Onstein, R. J. Carter, T. Stadler, and H. Peter Linder. 2014. Fossils and a large molecular phylogeny show that the evolution of species richness, generic diversity, and turnover rates are disconnected. Evolution **68**:2821-2832.
Xu, T. and M. F. Hutchinson. 2011. Anuclim Version 6.1 User Guide. The Australian National University Fenner School of Environment and Society., Canberra, A.C.T., Australia.
Yates, M. J., G. Anthony Verboom, A. G. Rebelo, and M. D. Cramer. 2010. Ecophysiological significance of leaf size variation in Proteaceae from the Cape Floristic Region. Functional Ecology **24**:485-492.
Yoder, J. B., E. Clancey, S. Des Roches, J. M. Eastman, L. Gentry, W. Godsoe, T. J. Hagey, D. Jochimsen, B. P. Oswald, J. Robertson, B. A. J. Sarver, J. J. Schenk, S. F. Spear, and L. J. Harmon. 2010. Ecological opportunity and the origin of adaptive radiations. J. Evolution. Biol. **23**:1581-1596.
Zachos, J., M. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science **292**:686-693.

Zachos, J. C., G. R. Dickens, and R. E. Zeebe. 2008. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. Nature **451**:279-283.

Zanne, A. E., D. C. Tank, W. K. Cornwell, J. M. Eastman, S. A. Smith, R. G. FitzJohn, D. J. McGlinn, B. C. O'Meara, A. T. Moles, P. B. Reich, D. L. Royer, D. E. Soltis, P. F. Stevens, M. Westoby, I. J. Wright, L. Aarssen, R. I. Bertin, A. Calaminus, R. Govaerts, F. Hemmings, M. R. Leishman, J. Oleksyn, P. S. Soltis, N. G. Swenson, L. Warman, and J. M. Beaulieu. 2014. Three keys to the radiation of angiosperms into freezing environments. Nature **506**:89-92.

Zhang, J.-B., R.-Q. Li, X.-G. Xiang, S. R. Manchester, L. Lin, W. Wang, J. Wen, and Z.-D. Chen. 2013. Integrated fossil and molecular data reveal the biogeographic diversification of the Eastern Asian-Eastern North American disjunct Hickory genus *Carya* Nutt.). PLoS ONE **8**:e70449.

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OTHER THESIS RESULTS

During my PhD I have collaborated with several people on projects related to the topics presented in this thesis. Although these projects are not the main focus of my thesis, I have included the publications, abstracts, and my contribution to these studies below.

Schwery, O., **Onstein, R.E**., Bouchenak-Khelladi, Y., Xing, Y., Carter, R., Linder, H.P. (in press). "As old as the mountains: the radiations of the Ericaceae". *New Phytologist.* doi: 10.1111/nph.13234.

Abstract. Mountains are often more species-rich than lowlands. This could be the result of migration from lowlands to mountains, of a greater survival rate in mountains, or of a higher diversification rate in mountains. We investigated this question in the globally distributed family Ericaceae, which includes c. 4426 species ranging from sea level to > 5000 m. We predict that the interaction of low specific leaf area (SLA) and montane habitats is correlated with increased diversification rates. A molecular phylogeny of Ericaceae based on rbcL and matK sequence data was built and dated with 18 fossil calibrations and divergence time estimates. We identified radiations using bamm and correlates of diversification rate changes using binary-state speciation and extinction (BiSSE) and multiple-state speciation and extinction (MuSSE) analyses. Analyses revealed six largely montane radiations. Lineages in mountains diversified faster than nonmountain lineages (higher speciation rate, but no difference in extinction rate), and lineages with low SLA diversified faster than high-SLA lineages. Further, habitat and trait had a positive interactive effect on diversification. Our results suggest that the species richness in mountains is the result of increased speciation rather than reduced extinction or increased immigration. Increased speciation in Ericaceae was facilitated by low SLA.

Author contribution:

I co-supervised O. Schwery during his MSc thesis at the Institute of Systematic Botany, at the University of Zurich. His MSc work has resulted in this publication. I have contributed by co-developing the project, by teaching O. Schwery how to conduct the analyses as well as selecting the functional traits and developing the trait measurement protocol for this study. I further contributed by providing comments on the manuscript. I was also responsible for our collaboration with the TRY database members, for which I had to design a research proposal to request data-sharing.

Breitkopf, H., **Onstein, R.E.**, Cafasso, D., Schlüter, P.M., Cozzolino, S. (in press). "Multiple shifts to different pollinators fuelled rapid diversification in sexually deceptive Ophrys orchids". *New Phytologist.* doi: 10.1111/nph.13219.

Abstract. Episodes of rapid speciation provide unique insights into evolutionary processes underlying species radiations and patterns of biodiversity. Here we investigated the radiation of sexually deceptive bee orchids (Ophrys). Based on a time-calibrated phylogeny and by means of ancestral character reconstruction and divergence time estimation, we estimated the tempo and mode of this radiation within a state-dependent evolutionary framework. It appears that, in the Pleistocene, the evolution of *Ophrys* was marked by episodes of rapid diversification coinciding with shifts to different pollinator types: from wasps to *Eucera* bees to *Andrena* and other bees. An abrupt increase in net diversification rate was detected in three clades. Among these, two phylogenetically distant lineages switched from *Eucera* to *Andrena* and other bees in a parallel fashion and at about the same time in their evolutionary history. Lack of early radiation associated with the evolution of the key innovation of sexual deception suggests that *Ophrys* diversification was mainly driven by subsequent ecological opportunities provided by the exploitation of novel pollinator groups, encompassing many bee species slightly differing in their sex pheromone communication systems, and by spatiotemporal fluctuations in the pollinator mosaic.

Author contribution:

For the study by Breitkopf *et al*. I conducted the orchid and *Ophrys* dating analyses and all diversification rate analyses. I contributed to the manuscript by writing the respective parts for the Materials and Methods and Results sections, and commented on the remaining text.

Xing, Y, **Onstein, R.E.**, Carter, R.J., Stadler, T., Linder, H.P. (2014). "Fossils and a large molecular phylogeny show that the evolution of species richness, generic diversity and turn-over rates are disconnected." *Evolution* 68:2821-2932. doi: 10.1111/evo.12489

The magnitude and extent of global change during the Cenozoic is remarkable, yet the impacts of these global changes on the biodiversity and evolutionary dynamics of species diversification remain poorly understood. To investigate this question, we combine paleontological and neontological data for the angiosperm order Fagales, an ecologically important clade of about 1370 species of trees with an exceptional fossil record. We show differences in patterns of accumulation of generic diversity, species richness, and turnover rates for Fagales. Generic diversity evolved rapidly since the Late Cretaceous and peaked during the Eocene or Oligocene. Turnover rates were high during periods of extreme global climate change, but relatively low when the climate remained stable. Species richness accumulated gradually throughout the Cenozoic, possibly at an accelerated pace after the Middle Miocene. Species diversification occurred in new environments: Quercoids radiating in Oligocene subtropical seasonally arid habitats, Casuarinaceae in Australian pyrophytic biomes, and *Betula* in Late Neogene holarctic habitats. These radiations were counterbalanced by regional extinctions in Late Neogene mesic warm-temperate forests. Thus, the overall diversification at species level is linked to regional radiations of clades with appropriate ecologies exploiting newly available habitats.

Author contribution:

The study by Xing *et al.* was developed in a collaborative fashion between all authors, and I further contributed by performing the diversification rate analyses and writing parts of the manuscript.

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